Reproductive System Infections in Women: Lower Genital Tract Syndromes

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Abstract

Gynecological and obstetrical infectious diseases are an important component of women's health. A system approach to gynecological and obstetrical infection helps unify and classify microbial etiology and pathogenesis within a clinical anatomical framework of lower and upper genital tract syndromes. The reproductive system of women includes the vulva, vagina, cervix, uterus, fallopian tubes and ovaries. During pregnancy additional tissues include the chorioamnion and placenta together with the fetus and amniotic fluid. We review in two parts reproductive system infection syndromes in women using selected research results to illustrate the clinical utility of the system approach in terms of diagnosis, treatment and prevention. We conclude that a reproductive system perspective will lead to improvements in understanding, management and prevention of these diseases.

Importance of Reproductive System Infections

Women's reproductive health is at the core of medicine and public health. The health of populations is a direct correlate of infant survival and women's health is the major determinant of infant health[1]. Differences in disease risk between men and women and among women are determined by biologic differences such as in sex steroid hormones, anatomy, host defenses, genetic variation and the effects of reproduction. These differences interact with external influences on psychological development, sociocultural environment and economic status to generate differences in disease risk among men and women[2].

Reproduction determines the evolution of *Homo sapiens* with most of the burden of reproduction carried by women[3]. Infections of the reproductive system profoundly impact the demographic properties of populations by differential impacts on fertility and infant survival[4]. For instance both epidemiologic studies and mathematical modeling demonstrate that reproductive system infection due to *Neisseria gonorrhoeae* and *Chlamydia trachomatis* dampen population growth in

areas where these infections are common and untreated[5, 6]. Such demographic influences impact human evolution and shape host defences. As examples sexually transmitted diseases may have fostered monogamy in human communities and menstruation may have evolved as a defence against pathogens that breach the cervical barrier to enter the endometrial cavity[4, 7]. Furthermore adaptive and innate immunity may have been shaped by natural selection to reduce inflammatory damage to key structures such as the fallopian tubes. Many epidemiologic studies have reported both HLA and Toll-like receptor allelic variants that correlate with risk of pelvic inflammatory disease and its sequelae[8-12]. Specifically polymorphisms in the genes encoding IL-12 β, IL-10 and TNF α are linked with tubal infertility due to *Chlamydia trachomatis*[13, 14]. Although women with strong immune responses may be more likely to survive infection their reproductive effectiveness may be less successful than women who are not as responsive. Thus the range of immune variability seen in populations may be in part due to the effects of alternative phenotypes on inflammatory responses to reproductive system infections in women[15].

To understand the clinical syndromes produced by infections of the reproductive system it is helpful to have an understanding of the immunology of the reproductive system in women and of the epidemiology of sexually transmitted diseases.

Immunology

Infection can spread along the epithelial surfaces from the lower genital tract through the cervix to the upper genital tract or through the blood system to involve the entire reproductive system and fetus. Topologically the reproductive system of women is open to the external environment and numerous host defence mechanisms are in place to prevent acquisition and canalicular spread of infection. Such host defence mechanisms include low pH in the vagina due to a healthy Lactobacilli microbiome, cervical mucous microstructure that is impenetrable to

microbial transit except at mid cycle, during menstruation or post birth. Throughout the reproductive system the molecular components of the Toll-like receptor innate defence system are expressed on epithelial cells which when stimulated secrete pro-inflammatory cytokines. As an example infection of epithelial cells by pathogens such as *Chlamydia trachomatis* result in the secretion of pro-inflammatory cytokines[16].

The adaptive immune system of the female reproductive system is linked to the systemic immune system at the induction phase of an immune response and to the common mucosal immune system at the effector phase. Anatomically the female reproductive system lacks organized lymphoid tissue as found in the gastrointestinal tract and antigen encounter between antigen presenting cells and naïve lymphocytes occur in regional lymph nodes such as the iliac and inguinal lymph nodes. However, antigen primed lymphocytes home back to mucosal surfaces providing long-term protection or contributing to immunopathology as sub-mucosal tissue resident T and B cells[17]. As an example sub-mucosal tissue resident T cells can be readily isolated from the fallopian tubes of women with tubal infertility due to *Chlamydia trachomatis* infection and such T cells commonly secrete IL-10 when stimulated with *Chlamydia trachomatis* heat shock protein 60[18, 19]. Immune responses generated in response to reproductive system infection can also be detected in peripheral blood and are also correlated with immune phenotypes such as resistance to *Chlamydia trachomatis* reinfection[20, 21] or to fallopian tube damage post *Chlamydia trachomatis* infection[22, 23]. Immune responses generated at other mucosal surfaces such as the oropharynx may also correlate with resistance to infection in the upper reproductive system mucosal sites in women[24]. During pregnancy systemic cellular immune responses to microbial antigens are depressed putting pregnant women at heightened risk for reproductive system infection such as with *Candida albicans*[25]. Pregnancy also greatly alters the phenotype of upper genital tract infection. Pelvic inflammatory disease is rare in pregnant women because the choriamnion overlays the cervical mucous plug. Rather ascending infection manifests itself as chorioamnionitis which in turn is a major cause of preterm birth[26].

Sexually Transmitted Disease Epidemiology

In general reproductive system infection infections are either due to an intrinsic dysbiotic microbiome or to external pathogens most of which are sexually transmitted. A general model of sexually transmitted disease (STD) epidemiology is used to define control strategies[27]. STD epidemiology is determined by pathogen transmission as described by its reproductive number R_0. For a sexually transmitted infection to spread in a population its reproduction number needs to exceed one. In a fully susceptible population R_0 is determined by three parameters, each of which is the focus for pathogen specific control programs.

$$R_0 = \beta cD$$

β is the transmission probability given contact between a susceptible and infected person, D is the average duration of infection and c is the average contact rate between an infected and susceptible person. Condom promotion and vaccines target reducing β; case finding and antimicrobial treatment target reducing D; and behavioural messages target changing c. In the absence of an effective STD control program β and D are relatively stable characteristic for each pathogen; therefore the critical determinant of the reproductive number of an STD in a population is the frequency and pattern of sexual interaction between transmitter and new susceptible. Within a given population, the subset characterized by high rates of sex partner change is central to maintaining STDs within a population. This subset is found within an exceedingly small core of the population. Because STDs concentrate among individuals in the core, immune responses also concentrate in such individuals likely shaping the virulence and antigenic structure of STD pathogens[28]. Study of the antigen structure of STD pathogens has uncovered many of those molecules relevant to immunity and immunopathogenesis. In the case of *Chlamydia trachomatis* such studies have also uncovered molecules suitable for the development of a subunit vaccine[29].

Vaccines will be essential to STD control because of the extended duration of infection required to generate immune responses that clear infection and protect against reinfection[30]. The time to development of protective immunity after infection takes several months depending on the pathogen[31]. This phenomenon is particularly important for the bacterial sexually transmitted infections (*Treponema pallidum, Neisseria gonorrhoeae,* and *Chlamydia trachomatis*) because control programs based on detection and antimicrobial treatment reduce the average duration of infection and thereby reduce the probability of acquired immunity. This phenomenon which is clearly seen with *Chlamydia trachomatis* has been termed arrested immunity and contributes to the risk of reinfection[32]. Thus vaccines for the three major bacterial sexually transmitted infections will be essential to future public health programs to prevent these infections.

Clinical Syndromes

Women's reproductive system infections can be classified anatomically and involve the vulva, vagina, cervix, endometrium, fallopian tube, ovaries, chorioamnion, fetus, neonate and infant (Table 1). Reproductive system infections may be due to microbes due to microbes that arise from a dysbiotic vaginal microbiome or from exogenous pathogens that are sexually transmitted or via instrumentation of the reproductive system. The major microbial agents involved in reproductive system infections are shown in table 2.

A system perspective is a clinically useful approach to conceptualize gynecological and obstetrical infectious disease. While similar pathogens are involved in both gynecological and obstetrical infectious diseases the clinical syndromes differ between non-pregnant and pregnant women. This is most apparent in the upper genital tract syndromes. We summarize selected research in order to demonstrate the validity of the reproductive system approach and to highlight advancements in understanding reproductive system infectious diseases in women. The results are discussed in two parts. The first part focuses on the

clinical syndromes that define microbial diseases of the vulva, vagina and cervix. The second part focuses on clinical syndromes that define microbial diseases of the endometrium, fallopian tubes and ovaries and the chorioamnion, fetus, neonate and infant.

Vulva

Three different microbial syndromes are major causes of vulvar disease. These syndromes are genital ulcer disease, genital warts and vulvar vestibulitis. We discuss genital ulcer disease and vulvar vestibulitis in this section and defer genital warts to discussion of cervical neoplasia since genital warts and cervical cancer are both caused by human papilloma virus.

Genital Ulcer Disease

Treponema pallidum and herpes simplex virus are the major causes of genital ulcer disease worldwide. *Haemophilus ducreyi*, lymphogranuloma venereum and donovanosis are minor causes except in specific geographic areas. A substantial fraction of genital ulcer disease remains etiologically undefined. At a global level genital ulcer disease is the major co-factor facilitating the heterosexual transmission of human immunodeficiency virus (HIV) infection and control of *Haemophilus ducreyi* infection has been correlated with reduction in HIV transmission in Africa[33]. During pregnancy *Treponema pallidum* bacteremia (and HIV viremia) are the most important causes of fetal infection resulting in intra-uterine fetal death and stillbirth[34]. In the developed world heterosexual syphilis outbreaks in urban areas are extremely geographically clustered, linked to commercial sex work and to rising case rates of congenital syphilis[35, 36]. When control has been attempted with targeted mass treatment with oral azithromycin incomplete control followed by a re-bound in case rates was observed perhaps because of disruption in herd

immunity to syphilis[37]. Such findings reinforce the conclusion that a vaccine will be essential to syphilis control.

Vulvar Vestibulitis

Vulvar vestibulitis is a chronic relapsing vulvar pain syndrome (vulvodynia). Affecting 10-15% of reproductive age women, it is the most common cause of dyspareunia. Vulvar vestibulitis or localized provoked vulvodynia is the major cause of vulvodynia where the inflammatory pathology is localized to the vulvar vestibule. The vulvar vestibule is composed of non-keratinized stratified squamous epithelium embryologically derived from the endoderm and is contiguous with urethral and bladder epithelium. The histopathology of vulvar vestibulitis includes lymphocytic and plasma cell infiltration together with hyperplasia of pain sensory nerve fibres[38]. Epidemiologically vulvar vestibulitis is strongly associated with a history of recurrent vulvovaginal candidiasis and other causes of vaginal discharge. Clinically vulvar vestibulitis is associated with signs of mild vestibular inflammation in association with marked pain hypersensitivity to light touch of the vestibule with a cotton tipped swab. Most women with the syndrome have Lactobacilli dominant vaginal microbiome without findings of vulvovaginal candidiasis or other causes of vaginal discharge. However 30 to 40% of women with vulvar vestibulitis have cutaneous hypersensitivity on skin testing with *Candida albicans*[39]. This is rarely found among women without this syndrome. It is likely that microbial antigens from pathogens such as *Candida albicans* produce a long lasting contact hypersensitivity immune reaction that underlies disease pathogenesis. While treatment with topical glucocorticosteroids, cromolyn, lidocaine or longterm oral flucanozole are ineffective, most severe cases respond to surgical resection of the vestibular epithelium[40, 41]. Improved treatment and prevention of vaginal infections with pathogens such *Candida albicans* among other microbes in addition to reducing exposure to other possible environmental antigens will be essential to prevention of vulvar vestibulitis.

Vagina

Among reproductive age women a healthy vaginal microbiome composed of predominantly Lactobacilli and little or no obligate or facultative anaerobes provides defence against exogenous pathogens and promotes healthy pregnancy [42]. A Lactobacilli vaginal microbiome is promoted by estrogen maturation of glycogen containing squamous epithelial cells. A Lactobacilli microbiome lowers vaginal pH and together with other innate host defenses prevents entrance of potentially pathogenic microorganisms into the lower genital tract.

Disturbance in the healthy vaginal microbiome underpins vaginal dysbiosis or vaginal infection. The four major syndromic causes of vaginal dysbiosis or infection are bacterial vaginosis, desquamative inflammatory vaginitis, trichomoniasis and vulvovaginal candidiasis. Of the four microbial syndromes only trichomoniasis is unequivocally sexually transmitted although *Gardnerella vaginalis* may be transmitted sexually. All but vulvovaginal candidiasis is characterized by loss of vaginal Lactobacilli[42, 43].

Bacterial Vaginosis and Desquamative Inflammatory Vaginitis

Bacterial vaginosis and desquamative inflammatory vaginitis are two common vaginal discharge clinical syndromes (Figure 1)[42]. While clinically distinctive they share several common features. They are both a dysbiosis of the vaginal microbiome with loss of vaginal Lactobacilli. Whether a woman is colonized with *Gardnerella vaginalis* or not likely determines whether bacterial vaginosis or desquamative inflammatory vaginitis develops following loss of Lactobacilli. They are both associated with chorioamnionitis, preterm birth and pelvic inflammatory disease. They are both diagnosed with wet mount microscopy of vaginal fluid. They both respond to treatment with topical clindamycin but remain subject to frequent recurrence post-treatment.

Cervix

Two major clinical syndromes characterize microbial diseases of the cervix. These syndromes are mucopurulent cervicitis and cervical neoplasia.

Mucopurulent cervicitis

Mucopurulent cervicitis is clinically diagnosed at the bedside as mucopus in the cervical os or ex vivo on white tipped cotton swab with or without edema, erythema and friability in the contiguous area of endocervical columnar epithelium called ectopy (Figure 2). A junction zone between the stratified squamous epithelium of the vagina and the single cell columnar epithelial surface of the endocervix is established in fetal life and in the majority of young women is visible on the face of the ectocervix. During the early years following the onset of sexual activity and pregnancy this epithelial junction zone undergoes metaplasia and is ultimately replaced by stratified squamous epithelium over the entire face of the ectocervix. The major microbial causes of mucopurulent cervicitis are *Chlamydia trachomatis, Neisseria gonorrhoeae* and *Mycoplasma genitalium*[44-46]. *Chlamydia trachomatis* mucopurulent cervicitis often has endocervical mucopus together with tissue changes resulting in edematous, erythematous and friable ectopy. *Neiserria gonorrhoeae* mucopurulent cervicitis is more often associated with mucopus alone. HSV-2 can cause ulcerative cervicitis especially during primary infection when both ulcers and vesicles appear on the ectocervical face.

Before the introduction of *Chlamydia trachomatis* control programs most women with cervical *Chlamydia trachomatis* infection had clinically evident mucoprulent cervicitis[44]. After the establishment of control programs the prevalence of mucopurulent cervicitis among women with cervical *Chlamydia trachomatis* infection dramatically declined suggesting that the clinical manifestations of mucopurulent cervicitis depend on the duration of infection and

the acquired immune response. As more *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections are detected and treated the prevalence of subclinical infection rise. In the case of *Neiserria gonorrhoeae* the rise of subclinical infection has been correlated with the rise in gonococcal strains that exhibit severe nutritional deficiencies that produce non-inflammatory infection[47]. Phenotypic changes in *Chlamydia trachomatis* have not been detected among strains causing asymptomatic infection versus those causing symptomatic syndromes such as mucopurulent cervicitis suggesting that the disease manifestations among *Chlamydia trachomatis* infected women are determined primarily by the host rather than the organism.

The cytologic and histologic features of mucopurulent cervicitis and cervical infection with *Chlamydia trachomatis* and *Neiserria gonorrhoeae* are well characterized. On Papanicolaou stain *Chlamydia trachomatis* cervical infection correlates with the presence of activated lymphocytes, histiocytes (antigen presenting macrophages and dendritic cells) and atypical metaplasia of endocervical cells[48]. On biopsy of the cervix *Chlamydia trachomatis* is associated with secondary lymphoid aggregates and plasmacytic infiltration[49].

As mentioned when treatment of *Chlamydia trachomatis* infection became more commonplace, the prevalence of mucopurulent cervicitis and its correlation with *Chlamydia trachomatis* and *Neiserria gonorrhoeae* infection declined and presently, most cervical *Chlamydia trachomatis* infections are subclinical. Because of this and because of major advances in *Chlamydia trachomatis* diagnostics based on nucleic acid amplification tests and single dose treatment with azithromycin, emphasis in *Chlamydia trachomatis* control moved from clinical recognition to screening and treatment. Since randomized control trials demonstrated that screening and treatment of *Chlamydia trachomatis* infection in women reduced the risk of pelvic inflammatory disease, large-scale public health programs based on screening and treatment to prevent infertility were launched[50]. Evaluation of large-scale *Chlamydia trachomatis* control programs in two Canadian provinces, Manitoba and British Columbia demonstrated that screening reduced pelvic inflammatory disease rates and concentrated infection into geographic core areas[51-53]. However,

the effect of infection on case rates and pelvic inflammatory disease rates was paradoxical. During the early years of the control program, *Chlamydia trachomatis* case rates fell but in later years, case rates dramatically rose while pelvic inflammatory disease continued to fall[54]. Figure 3 shows these divergent changes in British Columbia. Reinfection rates also increased because of concentration of infection into geographic core groups[53].

We interpreted these data to suggest that the result of the *Chlamydia trachomatis* control program has been to shorten the average duration of infection and interrupt the evolving immune responses. Arresting immunity reduces herd immunity, increases susceptibility to reinfection and causes annual case rates to rise. Reducing the average duration of infection reduces the probability of spread of infection to the endometrium and fallopian tubes, thereby reducing pelvic inflammatory disease rates. Support for these conjectures come form observations that women who spontaneously clear *Chlamydia trachomatis* cervical infection are more resistant to reinfection than women who remain infected and are treated[55]. Randomized control trials show that *Chlamydia trachomatis* screening and treatment does not reduce case rates[56] and seroepidemiological studies show that *Chlamydia trachomatis* control programs result in reduced prevalence but increased incidence[57, 58]. Thus a vaccine will likely be essential to achieving *Chlamydia trachomatis* control. Outer membrane proteins such as the *Chlamydia trachomatis* major outer membrane protein and the polymorphic outer membrane proteins are major T and B cell antigens[59]. It seems probable that a successful *Chlamydia trachomatis* can be developed based on these proteins despite the fact that they respond to immune pressure by antigenic variation[60].

Cervical Neoplasia

The identification of human papilloma virus as the etiological agent of cervical cancer is the single greatest advance in women's health in the last decades of the twentieth century. The identification of the virus immediately led to the development of vaccines and molecular diagnostic tests which transformed cervical

cancer prevention. Distinct genotypes of human papilloma virus cause vulvar warts and cervical neoplasia. There are over 100 genotypes of genital human papilloma viruses of which 13 are high- risk genotypes capable of producing cancer. Commonly human papilloma virus types 6 and 11 cause genital warts and types 16 and 18 are the major causes of cervical cancer. Cancer causing genotypes of human papilloma virus encode oncogenic E6 and E7 viral genes that inhibit host cell tumour suppressor proteins p53 and Rb. Most women with high-risk cancer causing human papilloma virus infection do not develop cancer due to the development of immunity or the absence of facilitating cofactors. Human papilloma virus like particles engender immunity when used as vaccine. There are currently three types of human papilloma virus vaccines containing two, four or nine viral genotypes produced by two manufacturers. The success of the human papilloma virus vaccine has been extraordinary and heralds the possibility that human papilloma virus caused cancers can be eradicated globally.

Cofactors are involved in human papilloma virus oncogenesis even when infection occurs with a high risk genotype. Eliminating cofactors may also assist in preventing cervical neoplasia. *Chlamydia trachomatis* is one established cofactor that may increase oncogenesis by facilitating human papilloma virus integration into the host genome since *Chlamydia trachomatis* produces double strand chromosomal DNA breaks during intracellular infection[61, 62]. Dysbiotic vaginal flora with a loss of vaginal Lactobacilli is another possible cofactor that may promote human papilloma virus oncogenesis through an altered vaginal metabolome that changes gene expression in cervical epithelial cells[63, 64]. Polyamines produced during bacterial vaginosis strongly bind to host cell histones and may alter gene expression in human papilloma virus infected cells. Control of facilitating cofactors may also assist in the prevention of cervical oncogenesis.

Conclusion

Lower genital tract infection syndromes are both common and similar in their clinical features among non-pregnant and pregnant women. Pregnant women

may be at enhanced risk of infection because of depressed cellular immune responses. In the next part (part two) we discuss upper genital tract infection syndromes. This is where non-pregnant and pregnant women most differ in disease expression and where the reproductive system perspective is most evident.

Table 1: Classification of reproductive system infections in women.

Anatomical site	Clinical syndrome
Vulva	Genital ulcer disease
	Genital warts
	Vulvar vestibulitis
Vagina	Bacterial vaginosis
	Desquamative inflammatory vaginitis
	Trichomoniasis
	Candidiasis
Cervix	Mucopurulent cervicitis
	Cervical neoplasia
Endometrium, fallopian tubes and ovaries	Pelvic inflammatory disease
	Tubal infertility
	Ectopic pregnancy
	Ovarian cancer
Chorioamnion, fetus, neonate and infant	Chorioamnionitis and prematurity
	Clinical amnionitis
	Stillbirth
	Congenital infection
	Neonatal sepsis
	Ophthalmia neonatorum
	Infant pneumonia
	Laryngeal papillomatosis

Table 2: Microbial causes of reproductive system infections in women.

Endogenous	Exogenous	
	Instrumentation	**Sexually transmitted**
Prevotella	Group A Streptococci	*Treponema pallidum*
Bacteroides		*Neisseria gonorrhoeae*
Atopobium		*Chlamydia trachomatis*
Mobiluncus		*Haemophilus ducreyi*
Peptostreptococci		*Klebsiella granulomatis*
E. coli		*Mycoplasma genitalium*
Group B Streptococci		*Gardnerella vaginalis*
Staphylococcus aureus		*Trichomonas vaginalis*
Candida albicans		Herpes simplex virus
Ureaplasma urealyticum		Human papilloma virus
Mycoplasma hominis		Human immunodeficiency virus

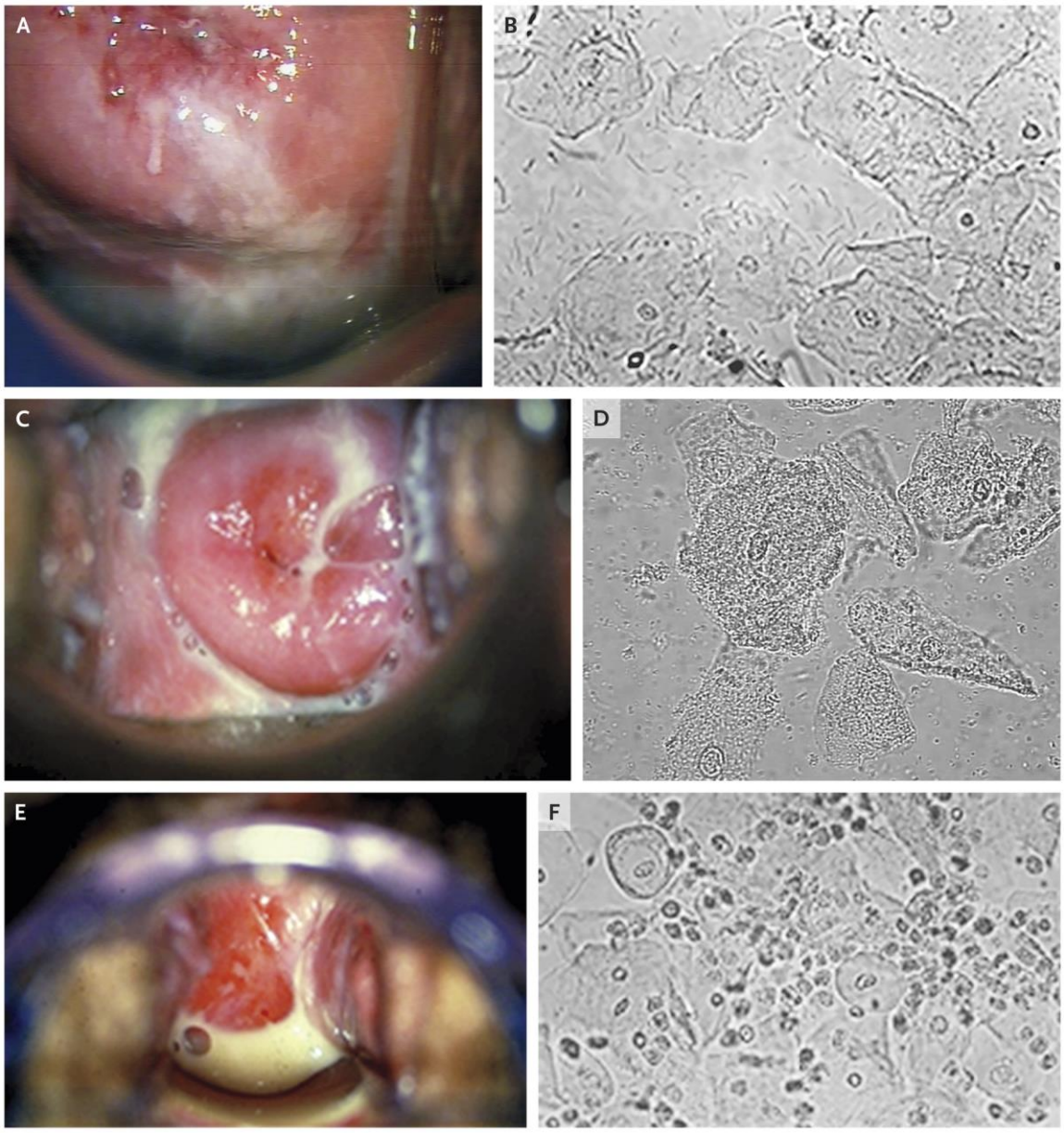

Figure 1: Features of healthy vaginal flora, bacterial vaginosis and desquamative inflammatory vaginitis. (A). Normal healthy cervicovaginal mucosa and small amount of vaginal discharge consistent with lactobacilli dominating. Note physiological cervical ectopy and clear cervical mucus. (B). Normal vaginal wet mount (400x). Note rod-like bacteria consistent with lactobacilli and lack of leukocytes. (C). Bacterial vaginosis. Note heavy milk-like homogenous vaginal discharge with bubbles, consistent with gaseous byproducts of anaerobic bacteria. (D). Wet mount findings of bacterial vaginosis. (400x) Note vaginal epithelial cells covered by coccobacilli consistent with clue cells. Note lack of leukocytes. (E). Desquamative inflammatory vaginitis. Note heavy yellowish vaginal discharge and inflamed cervicovaginal mucosa. (F). Wet mount findings of desquamative inflammatory vaginitis (400x). Note high number of leukocytes (mononuclear leukocytes dominate) and the presence of round parabasal cells consistent with inflammation[42].

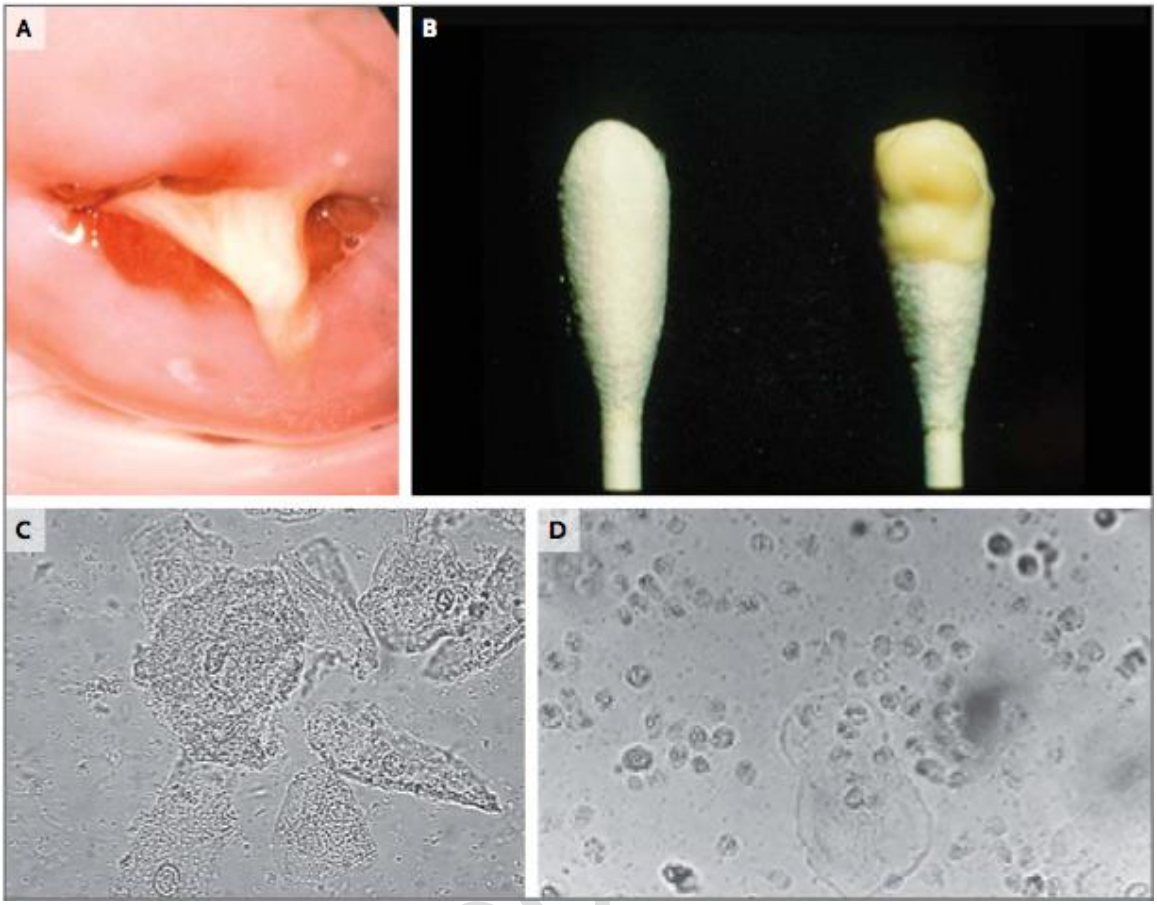

Figure 2: Diagnosis of pelvic inflammatory disease. The clinical diagnosis of pelvic inflammatory disease is based on the findings of pelvic tenderness on bimanual vaginal examination and of lower genital tract inflammation on speculum examination. Panel A shows mucopurulent endocervical discharge as seen on speculum examination. An area of endocervical columnar epithelium (ectopy) is seen on the face of the cervix. The epithelium is edematous and erythematous and bleeds easily when touched (friability). Panel B shows mucopurulent endocervical discharge as a yellow–green exudate on the tip of a Dacron swab (a positive swab test). Panels C and D show high-power microscopic examination of vaginal fluid, with clue cells typical of bacterial vaginosis (Panel C) and increased numbers of white cells (≥1 per vaginal epithelial cell) (Panel D)[65].

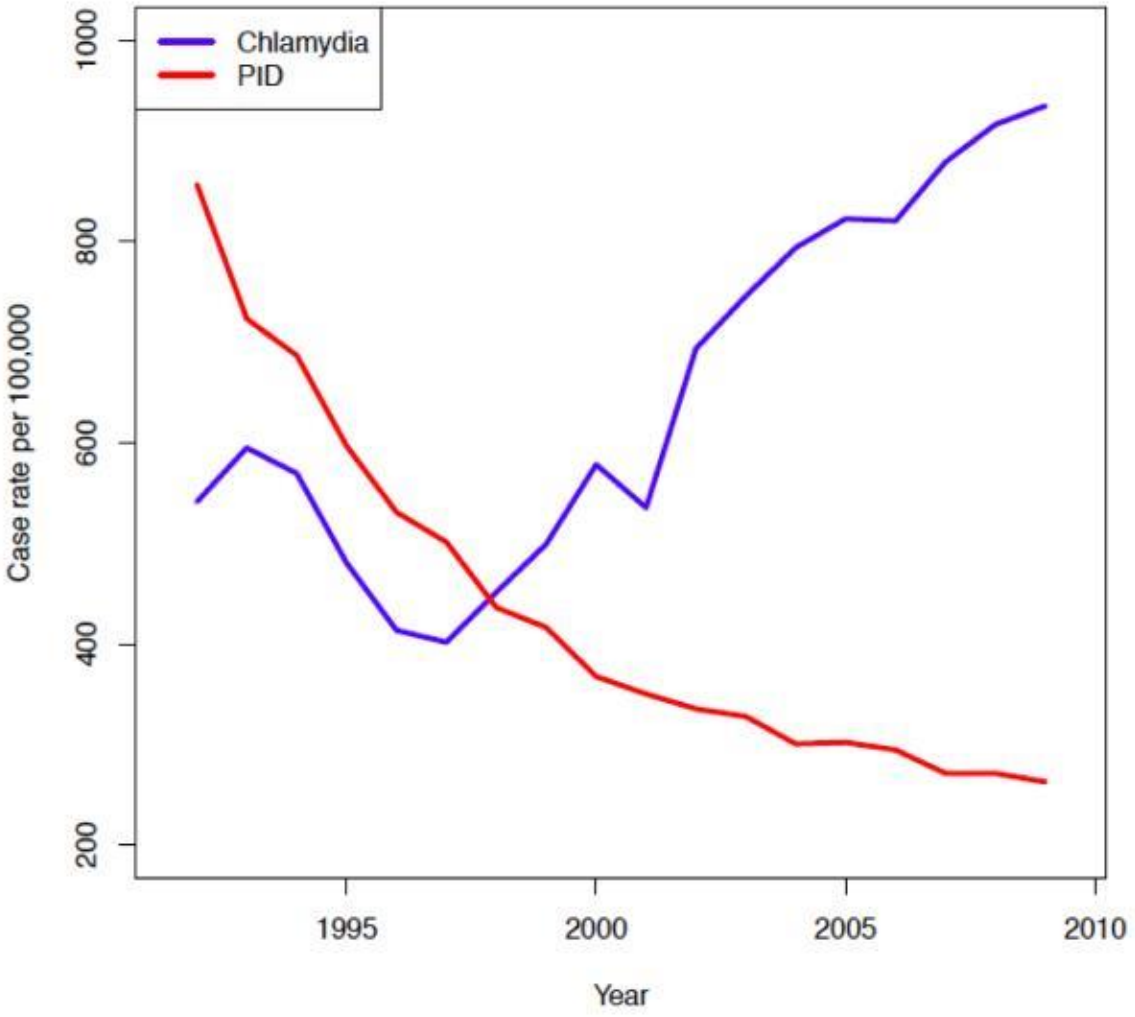

Figure 3: Shown are case rates for *C. trachomatis* infection (blue) among women between the ages of 15 to 39 years and clinical PID (red) among women between the ages of 14 to 44 in the province of British Columbia, Canada between the years 1994 and 2009[30].

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