Introduction

The cervix of the uterus is the anatomic border-line between the upper and the lower genital-tract in the female. It also represents a microbiological borderline, the vaginal flora consists of a wide variety of microorganisms, including aerobic and anaerobic bacteria, yeasts, mycoplasmas and viruses whereas the upper genital tract including the uterine cavity and the fallopian lubes in general, has long been regarded as sterile **(Moller et al., 1995).**

The cervix protects the uterine cavity from bacterial colonization. The cervical mucus is constantly produced and passes downwards into the vagina, both by ciliary action and gravity, thus washing out any invading bacteria. The alkaline pH of mucus may inhibit bacteria normally resident in the acid vagina. In addition, there is a local secretory immune system in the endocervix producing secretory_IgA which destroys bacteria in the presence of complement and lysosome, blocks bacterial adhesion to mucosal cells and promotes agglutination and phagocytosis **(Rebello et al., 1975).**

More than a century ago, Alfred **Doderlein (1892)** discovered that lactobacilli are inhabitants of the vagina, where they have a symbiotic relationship with their host. The vaginal flora is normally dominated by lactobacilli, which are responsible for reducing the pH by metabolizing glycogen from squamous cells to lactic acid.

The resultant acidic milieu provides protection against infection. A change in pH, possibly following menstruation or sexual intercourse, may be the trigger for Bacterial Vaginosis(BV) to develop. Women with BV are found to have a group of micro-organisms present in high concentrations. This group includes the anaerobic organisms *G.vagmalis, Mobiluncus, Bacteroides, Peptostreptococcus* species and *Mycoplasma hominis* and *Ureaplasma urealyticum.* There are a number of strains of some of these microorganisms (**Grattard *et al., l995*)**.

It may be that BV is a marker for bacteria endometriatis, but there is probably a poor correlation ' (**Keren and Spigland, 1978**). Bacteria have been identified in the endometrium in asymptomatic as well as symptomatic women **(Viniker, 1999).**

Aim of Work

A study of vaginal, cervical and uterine ecosystem in some gynecologic disorders.

Normal Genital Tract Ecosystem

The female genital tract is composed of a sequence of cavities. The external genital tract (vulva) leads into the vagina that connects in succession to the endocervix, the uterus and then to the Fallopian tubes. This passage allows for the fetus and menstrual flow to the exterior, and for the movement of spermatozoa to the interior. This exposure of the female genital tract to the external environment carries with it the risk of potentially compromising reproductive functions. Among the defense mechanisms that are operational in preventing infections in this area, undoubtedly one of the most important is the composition of the microbial flora that colonizes the vagina **(Witkin et al., 2007)**.

Historically, studies of the components of the vaginal ecosystem relied first on microscopic evaluation and then on identification of specific bacteria by culture techniques. However, utilization of culture media for the comprehensive identification of bacterial ecosystem diversity at a particular body site is now recognized as being incomplete and fragmentary. Culture-independent techniques have revolutionized bacterial detection. Application of the technique of amplification, cloning and subsequent sequence analysis of the genes encoding bacterial **16S** ribosomal **RNA** directly to vaginal samples has clarified the identification of the most common ***Lactobacillus*** species, demonstrated that lactobacilli are not always the dominant microbial species in apparently healthy women, and has identified previously undetected bacterial vaginal inhabitants **(Geraldo., 2007)**.

Studying the vaginal microflora is not only fascinating, with many discoveries to be made, it is also a very practical way to help women get rid of bothersome and sometimes dangerous infections- Gram-stained vaginal preparations, pap smears, specific cultures, and nucleic acid detection techniques can be used to diagnose the constituents of the vaginal flora, but in trained hands office-based microscopy of a fresh vaginal smear, preferably using a x400 magnification phase-contrast microscope.

The "**intermediate flora**" is addressed separately and a new entity – "**aerobic vaginitis**" - is discussed. Future research should focus on the interaction between infecting microorganisms and host defence mechanisms, as both together generate the pathogenicity of these conditions **(Donders, 2007)**.

Previous studies on the microbial flora of the human vagina indicate that micro-organisms normally present in the human vagina play a key role in preventing successful colonization by "**undesirable**" organisms, including those responsible for bacterial vaginosis, yeast infections, sexually transmitted diseases and urinary tract infections **(Donders et al.,2000)**.

Moreover, epidemiologic studies have clearly established that abnormal vaginal microbial communities and lower genital tract infections are significantly associated with an increased risk of HIV infection **(Martin et al., 1999)**. Based on this, researchers have speculated that the normal vaginal microbial ecosystem may play a pivotal role in lowering the probability of heterosexual HIV transmission **(Schwebke, 2001)**.

Clearly, an accurate understanding of the composition and ecology of the vaginal microbial ecosystem in normal healthy women is essential to understanding how the normal flora reduces the risk of acquiring these communicable diseases.

The vagina and its unique microflora form a finely balanced ecosystem, with the vaginal environment controlling the microbial types present and the microflora in turn controlling the vaginal environment **(Pybus and Onderdonk, 1999)**.

This ecosystem is dynamic with changes in structure and composition being influenced by age, menarche, time in the menstrual cycle, pregnancy, infections, methods of birth control, frequency of sex, number of sexual partners, as well as various habits and practices such as douching **(Burton and Reid, 2002)** and sexual behaviours.

In the past 100 years since the first microbiological study of the human vagina **(Doderlein, 1892)**, lactobacilli have been thought to be the predominant members of normal postpubartal vaginal microflora **(Antonio et al., 1999)**.

A diverse array of other bacteria such as Staphylococcus, Ureaplasma, Corynebacterium, Streptococcus, Peptostreptococcus, Gardnerella, Bacteroides, Mycoplasma, Enterococcus, Escherichia, Veillonella, Bifido-bacterium and Candida **(Marrazzo et al., 2002)** can be present but in much lower numbers.

It has been postulated that lactobacilli play a critical role in maintaining the normal vaginal ecosystem by preventing overgrowth by pathogens and other opportunistic organisms by producing lactic acid, hydrogen peroxide (H_2O_2), bacteriocins and other antimicrobial substances **(Hillier, 1998)**.

This could be because about 10 - 42% of women whose vaginal microbial communities lack appreciable numbers of lactobacilli apparently maintain "**normal**" vaginal ecosystems **(Marrazzo et al., 2002)**. Obviously microbial populations other than lactobacilli are dominant in a rather large proportion of normal vagina microbial communities, and alone or in some combination work to suppress the growth of pathogens.

However, the identity and diversity of these populations remain largely obscure and the complex interactions of the various members of the vaginal flora are still poorly understood **(Xia Zhou et al., 2004)**.

The use of non-culture gene amplification techniques has improved our understanding of the composition of the vaginal bacterial ecosystem. In most healthy women in the reproductive period the predominant vaginal bacteria are one or more of the following species of Lactobacillus: L crispatus, L Iners and L gosseri. However, in other apparently healthy women lactobacilli may be deficient or absent, being replaced by other lactic-acid-producing bacteria: Atopobium, *Megasphaera* and/or Leptotrichro species. Infection and/or proliferation of pathogenic bacteria in the vagina is suppressed by lactic acid production, bacteria-generated antimicrobial products, and the local activities of the innate and cell-mediated immune systems **(Giraldo et al., 2007)**.

Vaginal epithelial cells produce a range of compounds with antimicrobial activities. These cells also possess membrane-bound Toll-like receptors that recognize pathogen-associated molecular patterns. Recognition leads to pro-inflammatory cytokine production and antigen-specific immunity. Local production of IgG and IgA antibodies can also be initiated in the endocervix and vagina in response to infection **(Witkin et al., 2007)**.

LACTIC-ACID-PRODUCING BACTERIA

The dominant species in the vagina of healthy premenopausal women was initially identified as *Lactobacillus acidophilus*. This turns out to be an oversimplification, however. In healthy women with a lactobacillus-dominant vaginal microflora, the major phylotypes detected by gene amplification are *L.crispotus* and *L.iners* or *L.crispatus* and *L.gasseri* **(Fredricks et al., 2005)**. Additional species, *L.Jensenii*, *L.gallinarum* and *L.vaginalis*, have also been identified in some women.

A culture-independent investigation of vaginal lactobacilli in women from three continents reported that the dominant species were the same in each region: *L.crispatus*, *L.gosseri* and *L.Jensenii* **(Pavlova et al., 2002)**.

Most interestingly, apparently healthy vaginal ecosystems are maintained in some women in the absence of a lactobacillus-dominant vaginal microflora. *Atopobium vaginae* was identified as the dominant vaginal phylotype in one woman, and in two others appreciable numbers of *Megasphaera* species and/or *Leptotrichia* species were also reported **(Zhoux et al., 2004)**.

Atopobium, *Megasphaera* and *Leptotrichia* are all producers of lactic acid, similar to the lactobacilli. Therefore, the acidic environment of the vagina, recognized as an important defense mechanism against the proliferation of different microbial pathogens, can be maintained by bacterial species other than the lactobacilli. Possibly, when lactobacilli are unable to predominate in a particular vagina for whatever reason, another lactic-acid producing species fills this niche. *Megasphaera* and *Leptotrichia* are also capable of producing metabolites with an unpleasant smell. This renders detection of a vaginal odor in women lacking a lactobacillus-dominated vaginal flora as inconclusive evidence for the identification of a disease entity such as bacterial vaginosis, especially in asymptomatic women. Similarly, the variable morphology of *Atopobium*, from elliptical cocci to rod-shaped organisms occurring singly, in pairs, or in chains, makes its detection by Gram stain or wet mount problematic, and it is easily mistaken for other bacteria purported to be markers of bacterial vaginosis **(Verhelst et al., 2004)**.

A further complication to the comprehensive characterization of the vaginal microbial ecosystem is the presence of biofilms. Biofilms are colonies of microorganisms that adhere to and cover a solid surface; they can be identified on the surface of vaginal epithelial cells. Although most prominent in women with bacterial vaginosis where *Gardnerella* and *Atopobium* species predominate, the bacterial composition of vaginal biofilms in asymptomatic women still remains to be characterized **(Stevens Witkin et al., 2007)**.

Possible racial/ethnic differences in the composition of the "normal" microflora of the vagina have also not received appropriate research attention. The occurrence of hydrogen-peroxide-producing lactobacilli, purportedly active in antimicrobial defense, is lower in Black women. It has been reported that the vaginal PH of Black women is higher than that of White women among subjects who were not diagnosed as having bacterial vaginosis. Additional studies suggested that this difference was only statistically significant among women who had abnormal — i.e. non-Lactobacilli-dominated vaginal microflora. The enhanced prevalence of bacterial vaginosis as diagnosed by Gram stain in Black women as opposed to Whites might merely reflect an increased likelihood that bacteria other than lactobacilli typically predominate in the former population, and not that these women have an abnormal flora **(Lara Moreno et al., 2007)**.

FLUCTUATIONS IN VAGINAL FLORA

The composition of the vaginal ecosystem is not static but changes over time and in response to endogenous and exogenous influences. Variables include stage of the menstrual cycle, pregnancy, use of contraceptive agents, frequency of sexual intercourse, specific sexual partners, vaginal douching, use of panty liners or vaginal deodorants, and utilization of antibiotics or other medications with immune or endocrine activities **(Eschenbach et al., 2001)**.

Exposure to an altered milieu will cause a fluctuation in the local environment and heighten or diminish the selective advantage of specific vaginal microbes. For example, the loss of lactobacilli from the vagina has been associated with sexual intercourse or with the use of antibiotics for non-vaginal illnesses **(Schwebke et al., 1999)**.

Another study found that sexual intercourse without a condom had no effect on vaginal lactobacilli but led to elevated levels of *Escherichia coli* and facultative Gram-negative bacilli **(Eschenback et al., 2001)**.

Over the course of the menstrual cycle, vaginal levels of hormones and *glycogen* vary, and menstrual blood alters vaginal pH and provides a substrate for many microorganisms.

Nevertheless, levels of vaginal lactobacilli appear to remain constant throughout the cycle; non-Lactobacillus species increase during the proliferative phase, while Candida albicans concentrations are highest towards menstruation (as determined by culture) **(Eschenbch et al., 2000)**.

Since antibiotics can greatly alter the vaginal ecology, it is debatable whether women who are seemingly healthy and asymptomatic but deficient or lacking in lactobacilli on the basis of a wet mount microscopic examination should be treated. Inducing a perturbation in the endogenous microflora due solely to microscopic findings may lead to the selective proliferation of microorganisms that had been suppressed and that are detrimental to the individual woman's vaginal health **(Witkin et al., 1987)**.

The yeast C. albicans is tolerant of the acidic vaginal environment and is present in the vagina of approximately 10-20% of women of reproductive age. The concentration of this microbe is low, and carriage is typically asymptomatic. However, under conditions associated with a local immunosuppressive event - such as frequent sexual intercourse or induction of a local allergic response - *C.aibicans* can proliferate and also undergo morphogenesis to a more invasive hyphal form.[16] This results in the development of a symptomatic vaginitis **(Pavlova et al., 2002)**.

Regardless of the predominant bacterial species in the vagina of a healthy premenopausal woman, it appears certain that lactic acid production is crucial to the maintenance of a healthy vaginal ecosystem. The resulting acidic pH prevents the overgrowth of potentially pathogenic microorganisms. Additional benefits for the host of Lacto-bacillus predominance are production of hydrogen peroxide and bacteriocins by strains of these microbes **(Witkin et al., 2007)**.

Effect of estrogen on the vaginal environment

The composition of the vaginal microflora is believed to be influenced by the presence of estrogen: early investigations showed that estrogen stimulates the deposition of glycogen in the vaginal epithelial tissue. Glycogen is metabolized to glucose which is converted to lactic acid within the vaginal epithelium by cellular metabolism **(Stahl et al., 1986)**.

Evidence indicating the relation between the decrease in vaginal pH during childbearing period and glycogen content is supported by studies showing that women receiving estrogen and lack lactobacilli have a vaginal pH of 5.0-5.5 differing from the neutral vaginal pH. The vaginal epithelium becomes very thin in postmenopausal women, and glycogen is either absent, or its level is greatly reduced. The reduction in the glycogen level is believed to be at least partially responsible for the changes in vaginal PH and microbial flora in postmenopausal women **(Steger et al., 1978)**.

The effects of estrogen on vaginal microflora have been evaluated by studies done on prepubertal girls, women of childbearing age and postmenopausal women. The vaginal microflora, in prepubertal girls is characterized by predommance of anaerobic bacteria while that in women of childbearing age is predominated by facultative Lactobacilli, Gardnerella vaginalis and genital mycoplasmas they are also more frequently recovered from these women than from prepubertal girls **(Hillier et al., 1993)**.

Lactobacilli, yeasts and bacterial vaginosis-associated microorganisms are less frequently recovered from postmenopausal women "who do not receive estrogen replacement therapy" than from women of childbearing age **(Balows et al., 1991)**.

The use of estrogen replacement therapy result in increased vaginal colonization by yeasts, G.vaginalis, prevotella bivia and mycoplasma hominis, that may induce increased incidence of yeast vaginitis and bacterial vaginosis. But, increased colonization by lactobacilli and the resultant acidification of vagina may help offset of these risks **(Ginkel et al., 1993)**.

Hormonal changes may be important in the onset and clearance of bacterial vaginosis. Studied vaginal flora and serum oestradiol levels of 55 women at baseline and during hormonal treatment, none developed bacterial vaginosis (BV) from normal vaginal flora, 69% of women had normal flora at baseline increasing to 91% following hormonal treatment. The mean oestradiol level with BV was 39.07 ng/L compared with 176.41 nq/L with normal flora. Non-smokers had a mean oestradiol level of 173.95 ng/L compared with 118.67 ng/L in smokers. Recombinant follicle-stimulating hormone resulted in a mean oestradiol rise to 113.9 ng/L. The mean rise was 330.4 ng/L with improved vaginal flora but only 15.1 ng/L in persistently abnormal or worsening flora. A rise in oestradiol in