Old Herborn University Seminar Monograph

12. VAGINAL FLORA IN HEALTH AND DISEASE

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PRESS RELEASE:

TOP SCIENTISTS PUT OUT CALL TO ACTION ON WOMEN'S HEALTH

An assembly of some of the top scientists in the world specialising in bladder and reproductive tract infections in women called upon international granting agencies, government regulatory bodies and industry to act quickly to address issues critical to the health of around 1 billion of the world's female population.

Speaking in Herborn, Germany conference co-chairs, Dr. Philip Carter of North Carolina State University in the USA and Dr. Gregor Reid of Canada's Lawson Research Institute issued a wake up call to seek new and important methods to diagnose treat and prevent infections which afflict, debilitate and even kill women and their babies.

Reid said: "For too long, this area of human suffering has been down played, ignored or left to antibiotics and antifungals. As good as these drugs are, they have side effects. Organisms are developing resistance to them, but more importantly their use can increase the risk of another infection. Countries spend large amounts of money treating these infections and bearing the brunt of their economic and social impact instead of putting money into research, such as natural probiotics, to prevent infections."

Dr. Thomas M. Hooton, Infectious Diseases Specialist from Seattle, USA, showed how a simple bladder infection has a profound effect on a patient's well being and ability to work "When a patient experiences a bladder infection, she not only experiences pain and stress, but she often faces several days off work and the knowledge that her infection will likely recur within the next few months".

Dr. Sabina Cauci from Udine, Italy, reported on the critical bacterial factors which cause bacterial vaginosis. She, like her colleagues on the Herborn panel, is frustrated by the lack of funding in Europe, Canada, US and elsewhere to address important basic and clinical questions in this area.

Dr. David Taylor-Robinson from London, England reported on the growing problem of sexually transmitted disease especially Chlamydia. "These bacteria often cause devastating disease even without the woman knowing. Eventually it can cause ectopic pregnancy, pelvic pain and infertility."

Dr. Volker Rusch, Head of the Institute for Microecology in Germany said: "I am astounded at how little is understood about these prevalent bacterial and yeast diseases. I am even more astounded at the failure of scientific and government agencies to fund basic and clinical research when so many women want alternative therapies and to be better able to control their health maintenance."

Dr. Helen McDonald from Adelaide, Australia and Dr. Inger Mattsby-Baltzer from Göteborg, Sweden, showed how vaginal infections endanger the lives of

babies in the womb as well as increase the risk of premature birth. This is costly in terms of the life long well being of the new-born and in terms of the huge burden on the health care system. Swedish clinical expert Dr. Anders Hallen will soon commence a trial of lactobacilli probiotics to try and improve the outcome of vaginal candidosis.

There is now a clear association between depletion of the natural lactobacilli flora and an increased risk of

sexually transmitted diseases including AIDS. Dr. Carter said: "The impact of research on the urogenital tract has vital implications for many women. We ignore this at our peril."

The researchers will publish their work as well as a consensus document and make it available for scientists, consumers, industry and government decision makers.

Herborn-Dill, Germany
September 30, 1998

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Edward V. De Buysscher, D.V.M., Ph.D.
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THE ROLE OF THE VAGINAL FLORA IN HEALTH AND DISEASE

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SUMMARY

The urogenital tract of females is an important microenvironment which, for the most part, is free from infection for much of a person's life. While the introduction of sexually transmitted pathogens is an obvious means to give rise to infection, most cases occur due to organisms originating in the host. Three diseases, bacterial vaginosis (BV), candidiasis and urinary tract infections (UTI), cause many hundreds of millions of infections in the world's female population each year. Depending upon access to proper health care, cures can be achieved through use of antibiotics and antifungals. However, progress needs to be made to better understand how these infections develop. Given the microbial biofilms that exist in the vagina and the 50 or so species of microorganisms that inhabit this niche, the environment is complicated and ever changing. Bacterial interactions, symbiotic or otherwise, and external factors such as antibiotics, spermicides and hormones, can alter the composition and structure of the biofilm. With respect to key organisms which confer a degree of protection against pathogens, lactobacilli have been identified because of their prevalence and ability of some to express factors which inhibit pathogen adhesion and growth. If this knowledge is to be translated into therapeutic regimens, lactobacilli strains must be studied genetically, phenotypically and tested in the clinical setting. The opportunity to restore and maintain a normal flora using exogenous lactobacilli and by stimulating endogenous strains via perbiotic nutrients, is certainly worthy of exploration.

INTRODUCTION

The female urogenital tract is an orifice which is critical to reproduction and to disposal of urinary secretions. The outer skin region comprises the perineum or vulva inside which is the vaginal introitus and urethral opening. In close proximity is the anal opening to the intestine. This milieu is the habitat for a range of microbial species whose emergence comes via the intestine and food intake, the skin and through transfer from a male partner via sexual intercourse. Such a general and basic description, to some, may not appear necessary, but I believe it is critical if we are to truly understand the role of this complex flora in health and disease. While much emphasis has been placed upon the study of pathogens and their virulence factors, comparatively little is

known about the process of health restoration. Some fundamental questions remain to be answered: what is the "normal" flora, how do pathogens survive and emerge to infect the host, what role do the organisms originating in the urogenital tract play in human diseases?

This article will focus on the urogenital flora, particularly the vaginal flora of adults, and how it is influenced by factors other than sexually transmitted bacteria and viruses. The exclusion of sexually transmitted diseases should not be viewed as de-emphasising the enormity of the problem which afflicts hundreds of million women around the globe. The types of complications which result from an imbalanced flora will be discussed with primary focus on urinary tract infections (UTI), bacterial vaginosis (BV) and candidiasis.

WHAT IS NORMAL?

The urogenital flora is established at birth by contact with the mother's vagina at delivery and by intake of milk. This flora appears to be dominated by bifidobacteria and lactobacilli when the new-born receives human milk, and by *Enterobacteriaceae* and streptococci and *Bacteroides* in bottle fed infants (*Edwards*, 1993). The organisms which colonise at birth are referred to as primary and secondary colonisers. As the child grows to puberty, there is an association between oestrogen levels and lactobacilli. During a woman's reproductive phase, lactobacilli are at their peak counts in the vagina. Upon reaching menopause, it has been assumed that lactobacilli were depleted or absent, but a recent study of 73 women has shown that 49% have lactobacilli counts of 100,000 CFU/g of vaginal fluid (*Hillier* and *Lau*, 1997). Many dietary factors influence the colonisation of the intestine, such as casein/whey and phosphate content, oligosaccharides, lactoferrin, iron and proteins and indeed many of these components have now been included in artificial milk formulas. Furthermore, factors as yet unknown, based upon ethnicity of women, appear to influence vaginal colonisation such that higher levels of potential pathogens have been found in black women and lowest levels in Asian-Pacific Islander women (*Goldenberg* et al., 1996a). The intestinal microbial biofilms act as the major source of organisms which colonise the urogenital tract, thus making it important to better understand biofilm structure and function.

Importance of the biofilm concept in understanding the flora

The microbial biofilm is, I believe, a critical component in the balance between healthy or disease in the urogenital tract. Biofilms are composed of organisms in single and multiple layers surrounded by microbial and host matter particularly polysaccharides, in a structure that is invariably adherent to a surface. The first step in biofilm formation is the deposition of a host conditioning film onto cell surfaces. Vaginal epithelial cells are coated in conditioning films containing mucopolysaccharides, glycoproteins and other substances which can act as receptors for microorganisms. For example, Tamm Horsfall protein (THP) present in human urine and likely present in the vagina due to bathing of the area with urinary fluids, acts as a substrate for *E. coli* strains with mannose sensitive adhesins (*Hawthorn* et al., 1991). Another example is an association between elevated foetal fibronectin and BV (*Goffeng* et al., 1997; *Goldenberg* et al., 1996b) implying perhaps that BV organisms bind to the fibrinectin better than lactobacilli whose

numbers subsequently become reduced in the vagina of these patients.

Organisms first adhere to surfaces (including other bacteria already present on a vaginal cell) via physicochemical interactions described in the Derjaguin Landau Verway and Overbeck (DVLO) theory of colloidal stability (*Derjaguin* and *Landau*, 1941; *Verwey* and *Overbeek*, 1948). Basically, the theory describes two boundaries, the primary and secondary minima, which an organism must overcome on its approach towards a surface. At the secondary minimum, separation distances of >50 nm only attractive van der Waal's forces occur (*Busscher* and *Weerkamp*, 1987). At the primary minimum 10-20 nm both van der Waal's and repulsive electrostatic interactions (charge, hydrogen bonding and hydrophobicity) influence adhesion. Irreversible binding can occur when the separation distance is down to <1.5 nm and receptor-adhesin lock and key mechanisms are present. The practical issue here is to illustrate the complicated process of adhesion and the number of ways in which organisms can colonise a surface. Furthermore, it is easy to imagine how fluid alterations (because of hormonal and mucus changes, pH fluctuations, exposure to antimicrobials and semen etc.) can influence these interactions. Having stated that, it is quite remarkable that only about 50 species of organisms colonise the urogenital tract from an intestinal reservoir which contains over 400 species and a pool of 10^{14} bacterial cells (*Tancrede*, 1992). Therefore, there must be a degree of specificity involved in the colonisation and biofilm process.

Once adherent, the organisms growth and multiply and become integrated into a multi-species biofilm. In healthy reproductive aged women, this biofilm is dominated by lactobacilli, while in patients with BV, it is dominated by Gram negative rods, as will be discussed later. The structure of the biofilm is dynamic. While few ultrastructural studies have been carried out on the vaginal flora (*Sadhu* et al., 1989), much progress has been made in understanding biofilms. There is evidence from confocal scanning laser microscopy and differential interference contrast microscopy (*James* et al., 1995; *Sanford* et al., 1996; *Suci* et al., 1997) to show that they can have mushroom-like forms, separated by interstitial spaces filled with surrounding fluid (*Lawrence* et al., 1991; *Lewandowski* et al., 1995). The structure includes a linking film to the surface and a bulk area which can host anaerobic organisms (*Reid* et al., 1998b). The outer layer of the biofilm is the area exposed to host defences, antimicrobials and bacteria entering the system. There is now evidence from cell-to-cell studies which shows that bacteria within biofilms communicate with each other through quorum sensing signalling (*Davies* et al., 1998; *Kolter* and *Losick*, 1998). This might, in part, explain the ability of biofilms to alter metabolic functions and resist antibiotic and host defence attack.

Another feature of biofilms is symbiosis whereby the presence of one species positively affects that of another: for example the growth of BV organism *Prevotella bivia* leads to ammonia production which is utilised by *Gardnerella vaginalis* (*Pybus* and *Onderdonk*, 1997). Also, *P. bivia* produce sialidases which destroy mucins and enhance adherence of other BV organisms and impair the specific immunologlobulin A against the cytotoxin of *G. vaginalis* (*Cauci* et al., 1998).

Based primarily upon *in vitro* data, it is known that many interbacterial interactions take place. Lactobacilli have been shown to produce acids, bacteriocins, biosurfactants and hydrogen peroxide which effect the ability of potential pathogens to adhere, grow and dominate the flora (*Klebanoff* et al., 1991; *Reid* et al., 1998b). However,

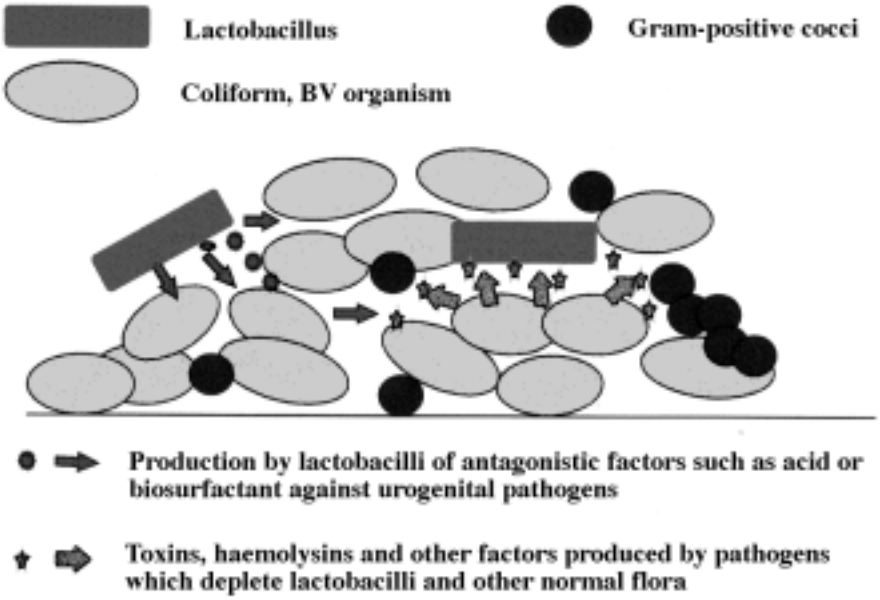

Figure 1: Microbial interactions within the urogenital flora.

there is significantly less evidence of this occurring *in vivo* and studies of this nature are needed.

What factors can influence the flora?

It has been assumed, and occasionally supported by scientific evidence, that urogenital disease occurs because pathogens express certain key virulence factors, such as toxins or haemolysins (*Hughes* et al., 1983; *Cauci* et al., 1996) (Figure 1). While such factors damage the host, the producers have to first to colonise the host (or at least find their way to bare cell surfaces) and presumably resist the competition of the existing flora (Figure 1). In the case of BV, the result is a significant depletion of lactobacilli and substantial increase in numbers of *Gardnerella* and other species causing the disease. Thus, there are competitive bacterial factors which over-ride the normal flora. One such factor is bacteriophage which could kill lactobacilli cells and thereby reduce their colonisation levels (*Kilic* et al., 1996). The extent of phage presence in the vagina and the ability of lactobacilli to resist their action is presently unstudied. Techniques now exist to label phages and follow their infection of bacterial cells within biofilms using scanning confocal laser microscopy (*Doolittle* et al., 1996) and such analysis would be very interesting in relation to urogenital biofilms.

External factors such as spermicidal agents, used more frequently because they can reduce the risk of STDs, deplete the lactobacilli flora especially strains producing hydrogen peroxide (*McGroarty* et al., 1990; 1992; *Cook* and *Rosenberg*, 1998; *Hira* et al., 1997). The effects of semen, douching and the use of oral contraceptives are less clear with respect to the vaginal flora. Certainly, semen increases vaginal pH, thereby reducing the effectiveness of acidity which kills viruses and inhibits the growth of some bacteria (*Kempf* et al., 1991; *Reid* and *Bruce*, 1995). There is new evidence to indicate that uropathogenic *E. coli* can be trans-

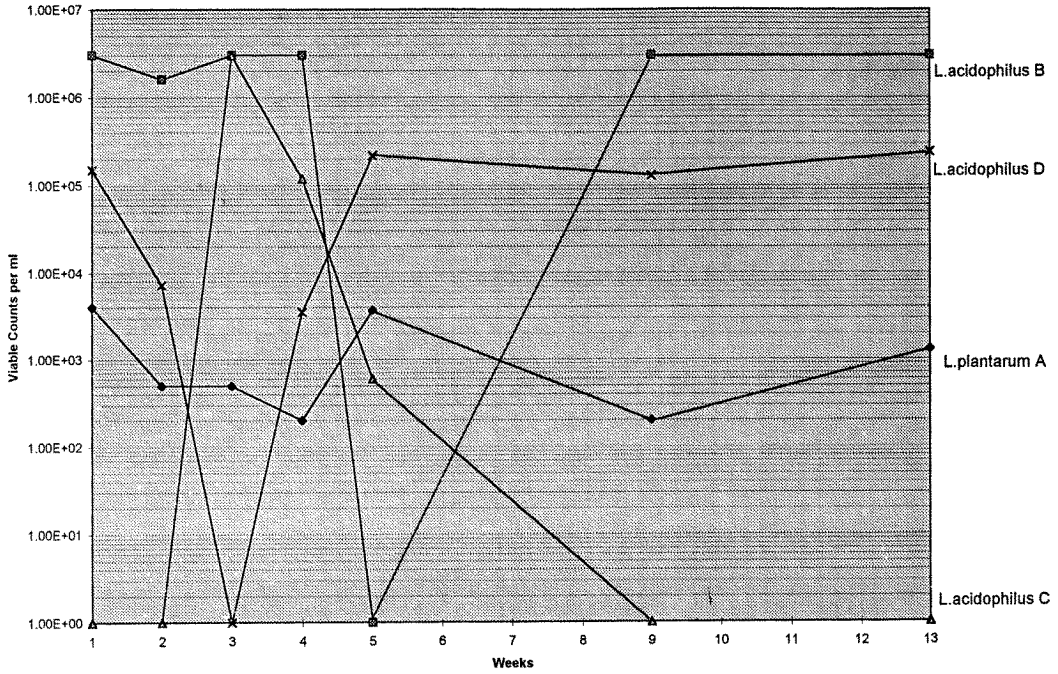

Figure 2: *Lactobacillus* strains from vagina.

mitted sexually (*Foxman* et al., 1997), making the recurrence of UTI a much more complicated scenario to understand and treat effectively.

Micturition has been shown to alter the flora on an hourly basis (*Seddon* et al., 1976), while reduction in oestrogen levels at menopause leads to lower lactobacilli counts and changes to the mucosal environment (*Raz* and *Stamm*, 1993).

The insertion of tampons, diaphragms and intrauterine devices (IUDs) can also disrupt the microflora. The classic example was the use of highly adsorbant tampons to which *Staphylococcus aureus* strains producing toxic shock toxins, attached and caused morbidity and mortality (*Berkley* et al., 1987). It is known that pathogen adhesion to IUDs can be associated with infections, however, when lactobacilli are the dominant organisms on IUDs, infections need not arise (*Reid* et al., 1988).

Once a urogenital infection ensues, the use of antimicrobial agents depletes the normal flora as well as the pathogens and thereby allows new primary and secondary colonisers to take over the microenvironment. The likelihood is that these will include drug resistance organisms, faster growing bacteria than the more fastidious lactobacilli, and intestinal coliforms. Antimicrobial agents are present in many food substances, and therefore the host is likely being exposed to their adverse effects on flora, on a long term basis.

The nutritional environment is clearly important for survival of members of the flora. Studies carried out on the colonic microflora showed conclusively that nutrient modulation can alter the composition of the flora (*Gibson* and *Roberfroid*, 1995). These studies have given rise to the concept of prebiotics which selectively modulate growth of non-pathogens in favour of pathogens. Recently, it has been shown that pre-

biotic compounds can influence the lactobacilli component of the vaginal flora without stimulating pathogens (*Reid* et al., 1995; 1998a). Certainly oligosaccharides have been shown to have utility in altering the flora composition of the intestine (*Tomomatsu*, 1994).

Another interesting feature of the dynamics of the microflora is that some strains appear to remain for a long time while others are transient. This has been shown in the intestine (*McCartney* et al., 1996) and more recently in the vagina (Figure 2). The latter findings as well as unpublished data from our laboratory, shows that new lactobacilli strains ascend into the vagina from the intestine. Perhaps the inability of some new strains to remain in the vagina reflects an inability to integrate into existing biofilms: if that holds true, it has implications for selection of probiotic strains that can survive long after insertion.

WHY ARE LACTOBACILLI IMPORTANT IN HEALTH MAINTENANCE?

There is now extensive evidence to support the claim that lactobacilli can be critical in the host's defence against urogenital infection (*Elmer* et al., 1996; *Hamilton-Miller*, 1997; *Hilton* et al., 1995; *Reid* et al., 1995; *Raz* and *Stamm*, 1993). In order to mimic the effect of the naturally occurring flora, it would appear to be rational to select strains with appropriate characteristics necessary to protect the host from infection. Some characteristics believed to be critical have been identified as early as 1984 (*Chan* et al., 1984; *Reid* et al., 1987, 1992; *Klebanoff* et al., 1991; *Velraeds* et al., 1996). The choice of strain type is perhaps less important although it should be from a species commonly recovered from the urogenital tract (*Reid* et al., 1996). The importance of strain selection has been highlighted by the finding that contents of health food products can be less than reliable (*Hughes* and *Hillier*, 1990) or with strains not named on the label (*Zhong* et al., 1998); very few are based upon lactobacilli expression of factors proven to be protective to the host.

Of all the characteristics which is the most critical? Arguments can be made for adhesion to be important, but factors which compete against other colonising organisms are also likely to be critical for health maintenance. The fact that strains possessing certain characteristics are present even in patients with recurrent infections, would imply that they are not vital in disease reduction. But some controversies exist, for example with hydrogen peroxide. Some studies show a correlation between H_2O_2 and prevention of BV (*Hawes* et al., 1996) while others show that H_2O_2 producers did not appear to protect against BV, UTI, vaginal candidiasis or trichomoniasis (*McGroarty* et al., 1992; *Rosenstein* et al., 1997).

In order to reduce the risk of UTI, it appears that a collection of factors are required including adhesion, production of growth and adhesion inhibitors and an ability to co-aggregate and form a balanced flora. Given the enormity of the microbial competition and the complexity of the host, it is likely that more than one lactobacilli strain will be required to maintain a normal flora.

A European based product, Gynoflor, reported to contain 50 mg of viable, H_2O_2-producing *L. acidophilus* and 0.03 mg oestriol has been tested on non-menopausal women with BV (*Parent* et al., 1996). This design is unusual in that it applies oestrogen to women who have no evidence of reduced oestrogen levels, and it uses

probiotics to actually treat infection rather than prevent recurrences. Six days of therapy with 1-2 vaginal suppositories per day gave a cure rate two weeks out of 77% in the treatment arm and 25% in placebo. The lactobacilli counts increased after treatment, although no DNA probe verification of the strain was used to confirm that it was the colonising organism. Nevertheless, the approach was successful. Likewise in a Japanese study of 11 women aged 20 to 60 years, intravaginal treatment with 5 ml commercial yoghurt reduced vaginal redness, lowered pH and caused a bacteriological cure (*Chimura* et al., 1995). Again, this is not a full proof study and it fails to provide light on mechanisms of action and properties of the probiotic strains (assuming that was what cured the patients), but it is another encouraging sign that this approach to therapy might work.

Delivery of lactobacilli to prevent urogenital infections has primarily involved direct insertion of the organisms into the vagina. However, there is new evidence to indicate that under certain conditions, it is possible to deliver probiotics to the vagina via oral intake (*Reid* et al., unpublished observations). Currently, the use of lactobacilli is arguably the best potential option to antibiotics for prophylaxis of UTI, but some vaccines are in development. A FimH-adhesin-based vaccine, delivery by systemic injection in animals shows promise (*Langermann* et al., 1997) but successful transfer to humans is a long shot and even then the limited target of type 1 piliated *E. coli* makes its' widespread use unlikely.

With respect to bacterial vaginosis, this is certainly a disease which affects a large population. It is defined as a mild infection of the lower female genital tract, characterised by the presence of three of five criteria: release of an amine (one or more of putrescine, cadaverine and trimethylamine)(fishy), release of an amine odour after addition of 10% potassium hydroxide, a vaginal pH greater than 4.5, clue cells in the vaginal fluid, and a milky homogenous vaginal discharge (*Amsel* et al., 1983; *Hillier*, 1993). The examination of clue cells consists of scoring the cell population as to being normal (0 to 3) and dominated by lactobacilli rods, intermediate (4 to 6) with colonisation by small gram-negative or gram-variable rods (*Gardnerella, Bacteroides* and possibly *Fusobacterium, Prevotella, Peptostreptococcus, Porphyromonas* and *Mycoplasma* species) and curved Gram-variable rods (*Mobiluncus*), and BV (7 to 10) with domination by the pathogens (*van der Meijden*, 1984; *Westrom* et al., 1984; *Spiegel* et al., 1980; *Cook* et al., 1989; *Nugent* et al., 1991; *Hillier*, 1993; *Holst* et al., 1994; *Rosenstein* et al., 1996).

There is a clear association between BV, reduced urogenital lactobacilli and increased risk of sexually transmitted diseases, including AIDS (*Biro* et al., 1995; *Cohen* et al., 1995; *Nilsson* et al., 1997; *Sewankambo* et al., 1997; *Paige* et al., 1998), and increased risk of preterm labour (*Chaim* et al., 1997; *Hillier* et al., 1995a; 1995b).

With respect to otherwise healthy pregnancy, the prevalence rates vary between 4.9% to around 7.5% (*Cristiano* et al., 1996; *Fan* et al., 1997; *Martinez de Tejada* et al., 1998) and perhaps vary between different socioeconomic populations and countries (*Llani-Camp* et al., 1996), such as Papua New Guinea where a prevalence of 23% has been reported (*Klufio* et al., 1995). A Danish study of 3,596 pregnant women showed a strong association between BV and *Gardnerella vaginalis, Mycoplasma hominis* and other anaerobes plus a depleted lactobacilli presence (*Moller*, 1998). Indeed, 59.6% of BV cases were associated with a combination of *G. vaginalis* and *M. hominis* again implying some sort of

symbiotic association.

In another study of 18 women over a 10 month period, BV was found to follow candidiasis in 9 of 11 episodes, to occur most often around the time of menstruation, and to resolve spontaneously in mid-cycle after unprotected sexual intercourse (Hay et al., 1997). These findings could be regarded as somewhat controversial and without a rationale, scientific explanation. They remain to be confirmed by others.

If an existing BV, isolated to the vagina, was solely responsible for increasing the risk of preterm labour, it would be expected that treatment of BV would have a favourable outcome on the pregnancy. This can occur (*Steele*, 1996) but it is not necessarily the case, in that metronidazole and clindamycin therapy for BV has been found not to reduce the preterm birth rate (*Joesoef* et al., 1995; *McDonald* et al., 1997). Failure, it could be argued, is due to the infecting bacteria having already ascended into the uterus and infected the foetus or endometrium: there is evidence to support this theory (*Hillier* et al., 1995a; 1996; *Peipert* et al., 1997; *Sweet*, 1995). Interestingly, a study which instilled a single 3% solution of H_2O_2 into the vagina of 23 patients with recurrent BV showed complete clearance of symptoms in 78%, thereby implying that this compound was important in prevention of BV (*Winceslaus* and *Calver*, 1996).

The presence of BV in pregnancy has been found to stimulate inflammatory cytokines in the vagina, in particular interleukin-1 beta (*Imseis* et al., 1997) although the role of these factors in pathogenesis, recurrence and onset of preterm labour remains to be clarified.

With respect to recurring attacks of *Candida* vaginitis, there does not appear to be an association between lactobacilli counts and infection risk (*Sobel* and *Chaim*, 1996). This finding does not rule out an association between onset of candidiasis because of a disrupted flora, nor an ability of lactobacilli with anti-*Candida* properties being able to reduce the risk of infection.

LACTOBACILLI AS THERAPEUTICS

The use of lactobacilli as a mono or mixed culture of live organisms, delivered as dried cells, a fermented product of douche, has been shown to benefit the host and is therefore an excellent example of a probiotic (*O'Sullivan* et al., 1992). As these authors illustrate, there are many so-called probiotic bacteria in commercial products, particularly in Europe and indeed an estimated 90,000 tons of probiotic yoghurt is produced in France each year. However, none of these probiotic organisms have been tested or shown to have any effect in maintaining a healthy urogenital tract which has a reduced risk of UTI. As the evidence provided above indicates, given the proper selection of lactobacilli strains, such therapeutic benefits are certainly possible (*Bruce* and *Reid*, 1988; *Bruce* et al., 1992; *Reid* et al., 1995).

The development of molecular biology tools has impacted upon lactobacilli, providing the potential to create strains which possess added probiotic properties, such as nisin a posttranslationally modified antimicrobial peptide widely used in the food industry as a preservative. Nisin-inducible expression cassettes have been transferred from the producer, *Lactococcus lactis* to *Lactobacillus helveticus* (*Kleerebezem* et al., 1997). In another example, studies have shown that lactobacilli have the potential to be a carrier for oral and perhaps vagi-

nal immunisation, say against *Chlamydia*, because of their adjuvanticity (*Pouwels* et al., 1996). This latter work has immense potential because lactobacilli are naturally occurring and they maintain viability in the vagina. On the negative side, systems will have to be put in place to control or shut down expression of antigens so as not to create the wrong type of host immune response such as anaphylaxis. In the end, it will be government regulatory agencies who will dictate the impact of some of these new developments. For now, most are unlikely to approve use of recombinant organisms and strains possessing plasmids for probiotic application to the human vagina.

The use of prebiotics, functional foods or health supplements, on the other hand, do have a real chance of being approved and made available. The opportunity will be to find the substances and strains of greatest effect in the urogenital tract. With market projections of US $ 100 billion by 2010, (*Stanton*, 1998) and increased scientific interest in the field, there should be an accelerated emergence of natural remedies for the healthy maintenance and restoration of the urogenital tract. Hopefully, this will be led by selection of organisms with proven scientific based characteristics and clinical efficacy.

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IMMUNE REGULATION OF THE FEMALE REPRODUCTIVE TRACT

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INTRODUCTION

Historically the reproductive tract has been considered a part of the mucosal immune system. The main common features are the secretion of IgA by the polymeric Immunoglobulin Receptor (pIgR), the presence of Intra Epithelial Lymphocytes (IEL's) and the fine discrimination between tolerance and response. However, the reproductive tract has equally common features with the central compartment of the immune system; e.g. the preponderance of IgG as the effector isotype in the vagina and the absence of an organised reproductive mucosa associated lymphoid tissue that samples and processes the antigenic contents of the lumen of the reproductive tract. Recently several excellent reviews have been written on the immune defence in general of the female genital tract (*Parr* and *Parr*, 1999), on the hormonal influence of the immune response (*Wira* et al., 1999 and on animal models to study immunoprophylaxis (*Hook* et al., 1999).

This short review should be considered as complementary to these reviews and aims to emphasise some unique features such as the maintenance of a homeostatic vaginal flora by immunoregulation, the cyclic impermeability of the vaginal epithelia for sperm- and other antigens and a change in local lymphocyte mediated cytotoxicity during gestation.

It is not possible to discuss the immune defence of the vagina independently of the rest of the reproductive tract. The products of the ovaries, oviduct, uterus and cervix contribute substantially do the immune status of the vagina. Unique to the immunobiology of the reproductive tract are its multiple and apparent contradictory functions, for example, the defence against potential pathogens while maintaining the beneficial (homeostatic) flora of the vagina. Following each mating/insemination the local vaginal-cervical and/or uterine environment is compromised. The disturbances in the homeostatic flora should be restored quickly to prepare for potential implantation of the embryo(s). Implantation and gestation require a very unique contribution of the immune system which results in allogeneic tolerance while maintaining an acceptable level of xenogeneic immunity. While the special immunology of gestation will not be discussed here, allogeneic tolerance mechanism have to be maintained against sperm components. This particular tolerance seems to result mainly from the temporary impermeability of the vaginal/cervical epithelium and the immunosuppressive effect of sperm components (*Bronson* and *Fusi*, 1999; *Tristram* and *Ogra*, 1999)

Unique also to the immunology of the female reproductive tract is the cyclicity regulated by the secretion of oestrogens and progestagens, which change both morphology and functionality of the different regions of the tract.

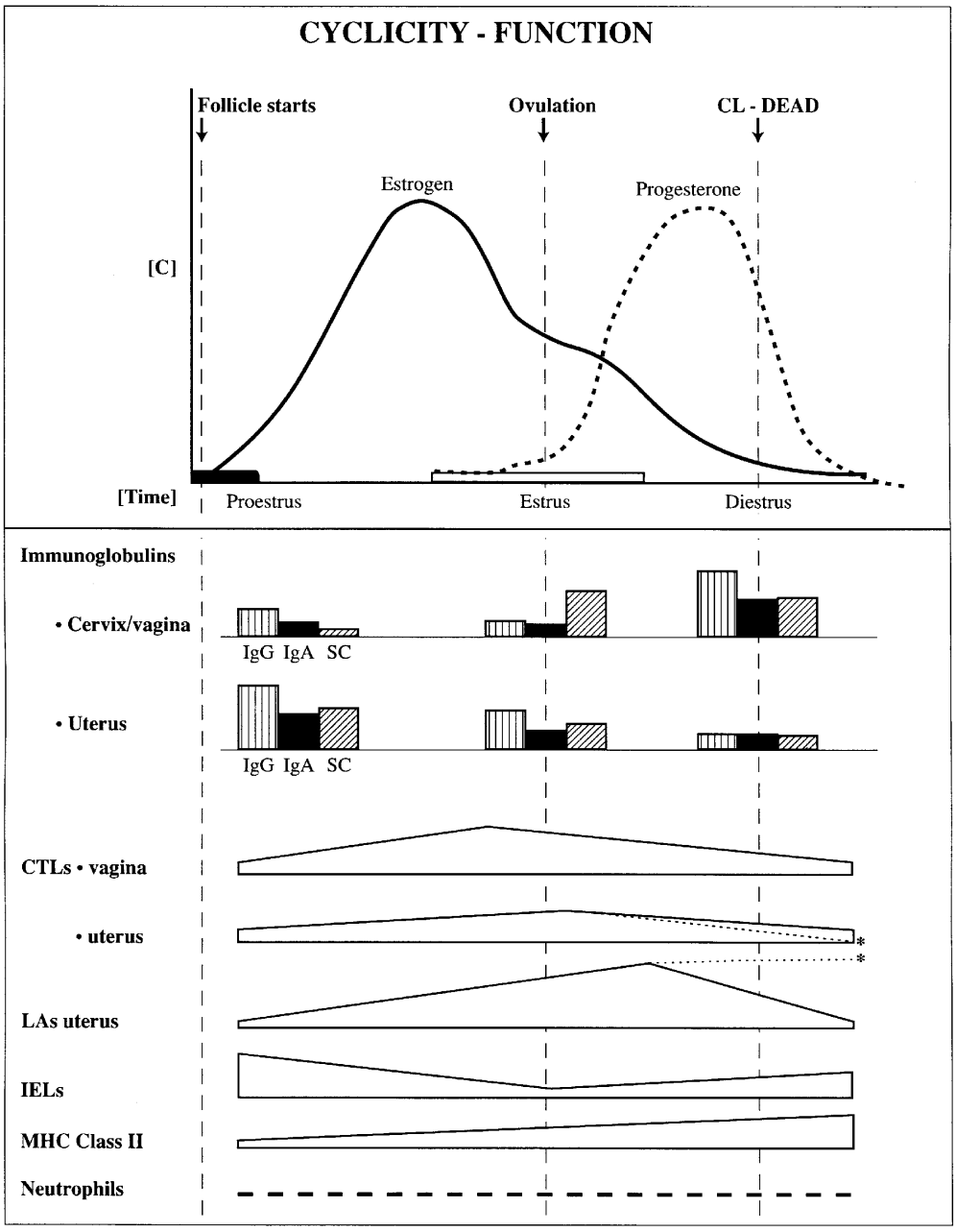

Figure 1: The relationship between the ovarian cycle and the relative abundance of immune components in the female reproductive tract.
Immunoglobulins: in general the secretions of the uterus are highest when they are lowest in the vagina.
SC (pIgR); secretory Component, this IgA transport molecule seems to be in excess in the non-inflamed reproductive tract.
CTL; Cytotoxic Lymphocyte. These cells peak around the time of ovulation. The CTLs disappear from the uterus when nidation and potential pregnancy is established (- - - - *).

Figure 1 depicts the ovarian hormonal cycle of a prototype mammal and the level of functionality, in the reproductive tract, of antibody isotypes and immune effector cells at different phases of the cycle. Very little data are available on the normal immune function of the vagina once gestation is established (*Tristram* and *Ogra*, 1999)

An additional source of complexity is the uniqueness of the reproductive strategy of the different mammalian groups, which is reflected in the different structures of the reproductive tract of the various species. The major experimental animals, the mouse and rat, have a somewhat similar copulation/insemination/fertilisation pattern. But even in this case it should be pointed out that the rat, unlike the mouse, has a very active entero-hepatic IgA recirculation and this might be the reason that hormone induced immunoglobulin secretion is preferentially studied in this species. Both genera utilise direct deposition of sperm to the cervix. In humans, by contrast, entero-hepatic IgA circulation is minimal, sperm is deposited outside the uterus and the cervix has a more active function in the uptake of the sperm cells. The structural result is that in the rat and mouse, vagina and cervix have a rather similar morphology and function, while in humans the cervix has become a centre of immune cells and antibody secretion (*Parr* and *Parr*, 1994). This is just one example of a different structure/function of the female reproductive tract among mammals. If one considers other groups such as swine, cows, horses and carnivores, it is not easy to reconstruct a common mammalian defence strategy of the reproductive tract. Figure 2 depicts the structure-function relationship of different parts of the reproductive tract of a prototype mammal.

Finally, we should mention the contribution of motility of the female reproductive tract to remove the ejaculate and hence the bulk of allogeneic and xenogeneic antigens. The importance of motility continues after fertilisation and little is known about its importance or connection with the immune system.

ORIGIN AND CONCENTRATION OF IMMUNOGLOBULINS IN VAGINAL FLUID

Ovary

The developing ovarian follicle produces a relatively large amount of follicular fluid. This fluid is very high in IgG (>10x serum concentration). The mechanism of this active transport is not very well studied, except that the IgG originates from the general circulation. Its function is also obscure and it is unknown if this fluid is largely taken up

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LA: Lymphoid aggregates; these tolerogenic structures are adjacent to developing endometrial glands. They increase towards the time of the arrival of the ovum (or zygote). When there is no pregnancy the LAs decrease towards the beginning of the next cycle. In case of pregnancy their number increases (- - - - *).

IEL; Intra Epithelial Lymphocyte.

MHC class II; as expressed on epithelial cells of vagina and cervix/uterus. An increase in class II expression is by some authors considered as an increase in antigen recognition and processing.. Peak class II expression occurs when the vaginal epithelium is thinnest and the access to the draining iliac lymph nodes is possible.

Neutrophils.; in the normal reproductive tract these cells are present in low numbers. However when luminal complement is activated. neutrophils can emigrate into the lumen in massive numbers. This luminal emigration can take place in a very short time period.

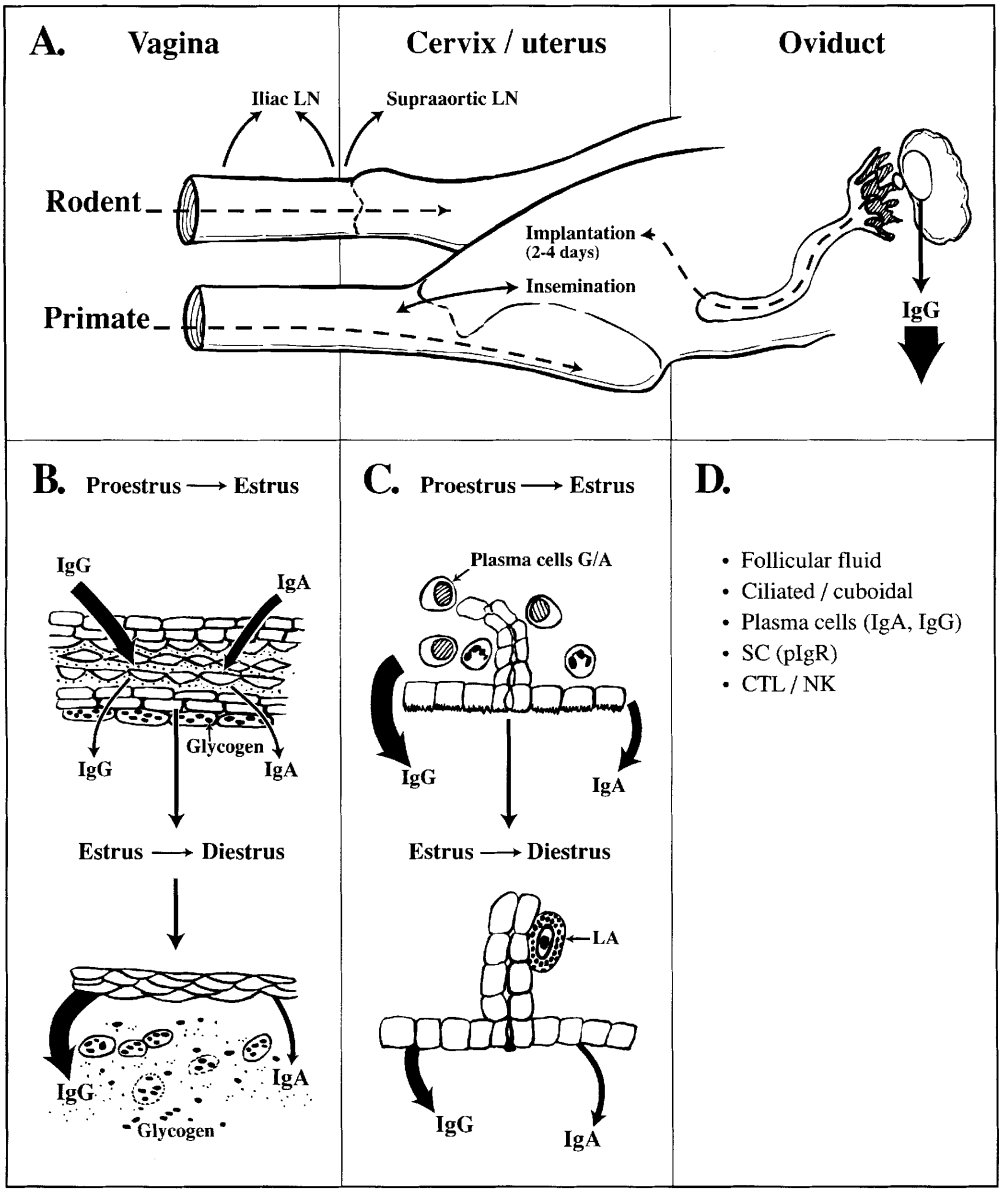

Figure 2: Structure-function relationship of the female reproductive tract.
A: Two different reproductive strategies are represented. Rodents with direct deposit of semen into the uterus and primates with deposition of sperm outside the uterus. As a result the immune components of the cervix are more pronounced in primates.
B: During the progression pro-oestrus oestrus, the squamous vaginal epithelium accumulates mainly IgG below the cells of the outer layers of the epithelium, these ageing cells also accumulate glycogen storage granules. During the progression from oestrus di-oestrus the outer layer of the epithelium is sloughed off, the cells disintegrate and allow the resident flora to expand utilising the released glycogen. During this expansion the local flora is selected for beneficial bacterial variants by the IgG that is released from the intercellular storage. At this time the vaginal epithelium is also very thin and now allows the draining of (bacterial) luminal antigens to the iliac lymph nodes.

by the infundibulum and serves as a defensive solution in which the ovum is transported to the uterus.

Oviduct

The data on the immune structures in the oviduct are few. It has been reported that the polymeric immunoglobulin receptor (pIgR) can be demonstrated on the apical sites of glandular and luminal epithelial cells. IgA plasma cells (although few) are present in the stroma and IgA and IgG can be demonstrated interstitially and in the apical regions of the epithelial cells. No data are available on the transport mechanism of IgG across the epithelium It is thought to be intracellular. Few data are available on the influence of the ovarian cycle on the immune parameters of the oviduct (*Wira* et al., 1999).

Uterus

During di-oestrus (high progesterone concentration) there is little contribution of immunoglobulins to the secretion from the uterus. When oestrogen increases during pro-oestrus there is a simultaneous increase in stromal IgG and IgA (*Richardson* and *Wira*, 1997). The increasing oestrogen concentrations enhance the transcription and translation of pIgR in glandular and luminal epithelial cells. Just before oestrus the secretion of sIgA and IgG is at its highest. At this time plasma cells (IgA) can be observed in the stroma, although not in the same amount as in the small intestine (*Wira* et al., 1999). At the time of oestrus this secretory phase has peaked, the expression of pIgR decreases with increased progesterone levels until the cycle is repeated after the disintegration of the corpus luteum. The main immunoglobulin in uterine secretions is IgG. The mechanism by which IgG reaches the apical site of epithelia is not well understood. Although IgA is generally lower than IgG, the uterus (and human cervix) are the main contributors of IgA in cervico-vaginal secretions.

Cervix

In rodents the epithelium of the ectocervix is similar in form and function to that of the vagina. In humans, however, both endo- and ecto-cervix act as major immune defence centres (*Parr* and *Parr*, 1999). The pIgR is under similar hormonal control as the in uterus. Some IgA and IgG plasma cells are observed in the cervix. While IgG is the predominant isotype in uterine secretions. The cervix and uterus contribute 90% of IgA that is found in cervico-vaginal secretions (*Kaushic* et al., 1997).

While some plasmacells are present in the normal, non inflamed tract, it is thought that the majority of secreted immunoglobulins is derived from the general circulation (*Hook* et al., 1999).

C: The epithelium of the cervix and uterus actively secretes IgG and IgA during the progression from di-oestrus pro-oestrus. These secretions reach the vaginal lumen at a time when immunoglobulin secretion by the vaginal epithelium is low.. During this time IgG and IgA plasma cells are most numerous as are granulocytes. During the progression oestrus di-oestrus immunoglobulin secretion is at its lowest in cervix and uterus, while it now reaches its peak in the vagina. At this time the Lymphoid Aggregates (LA) develop to prepare the uterus for its tolerogenic function in the event implantation takes place.
D: The ovary and oviduct also contribute to the secreted immunoglobulins, especially the follicular fluid).

The thickness of the arrows correlates with the relative abundance of the immunoglobulin isotypes.

Vagina

The timing of immunoglobulin secretion by the vaginal epithelium is basically complementary to the secretions by the uterus. When immunoglobulin secretion by the uterus is high during pro-oestrus (high oestrogen) the secretion is low in the vagina (*Kaushic* et al., 1994). The expression of pIgR follows the same pattern during pro-oestrus low in the vaginal epithelium and high in the uterine epithelium (*Wira* et al., 1999). When di-oestrus is reached (high progesterone) the sequence reverses and the concentration of IgG and IgA reaches a peak in the vaginal fluid while reaching its nadir in uterine secretions (*Parr* and *Parr*, 1994; *Parr* and *Parr*, 1999). This sequential timing of secretions gives the apparent picture that after mating the uterine environment is cleared of potential harmful antigens, first in the uterus followed in sequence by the immune clearance of the vagina. IgG is also the major isotype in vaginal secretions and the relative concentration of IgA is substantially less than in uterine secretions (*Parr* and *Parr*, 1999; *Rosenthal* and *Galichen*, 1997). Therefore the excess expression of pIgR might represent extra-transport capacity to be used during inflammation when blood supply and local plasma cell numbers increase, allowing for the rapid transport of the extra IgA The mechanism by which IgG reaches the vaginal lumen through the multilayer squamous epithelium is thought to occur in two phases (*Parr* and *Parr*, 1994). First IgG penetrates the basal layers via intercellular channels and accumulates below what is described as the granular layer. This granular layer consist of the top few layers of epithelial cells. These luminal cells contain large glycogen storage granules that are synthesised under increasing oestrogen concentrations. These cells are sloughed, release their glycogen that is converted to lactic acid by lactobacilli to create the optimum pH microconditions for the homeostatic vaginal flora. Simultaneously the IgG stored below the glycogen rich cells is now released and can act to neutralise pathogens but also contribute to the selection of the most beneficial of the vaginal flora in a manner described for the intestinal bacterial homeostasis by Rolf Freter many years ago (*Freter*, 1974). This time point coincides with the minimum thickness (layers) of the vaginal epithelium, the highest expression of Class II and permeability for non-self molecules (*Wira* and *Rossoll*, 1995) that can reach either actively or passively the draining iliac lymph node (*Prabhala* and *Wira*, 1995). This is the only time during the cycle that the vaginal epithelium is permeable for foreign antigens. It is also the time point when experimental infections with *Chlamydia* (*Kaushic* et al., 1998) and HSV-2 can be established successfully in rodents (*Parr* and *Parr*, 1998; *King* et al., 1998). It is apparent that any attempt to vaccinate via the intra vaginal route should take place during this phase of the cycle. Preliminary experiments indicate that during this phase foreign lymphocytes can penetrate the thin squamous epithelium and drain to the iliac node (*King*, 1998; *Rosenthal* and *Gallichan*, 1997). This observation lends credibility to the "Trojan Horse" hypothesis for heterosexual HIV transmission in the non-inflamed genital tract (*Ibata* et al., 1997; *Hook* et al., 1999; *Yeaman* et al., 1998).

DISTRIBUTION AND FUNCTION OF T-CELLS

T-cells are randomly distributed throughout the genital tract. They tend to be more numerous in the human cervix and rat vagina (*Givan* et al. 1997). A

linear correlation seems to exist between the number of T-cells and rising oestrogen levels (*White* et al., 1997a). However, this might merely reflect an increase in perfusion and total tissue mass of the tract. Of greater importance is their activity. When the total cytolytic activity of isolated T-cells is measured *in vitro* (CTL's), using an anti-CD3 monoclonal Ab, moderate cytolytic activity is found during the pre-secretion phase (high oestrogen-low progesterone) of the cycle (*White* et al., 1997b). It has been proposed by some investigators that these CTL's are important in inducing the secretory phase of the endometrium. However, once the secretory phase of the endometrium is reached (high progesterone-low oestrogen: oestrus => di-oestrus) the cytotoxic activity drops to non-detectable levels (*White* et al, 1997b). These observations also corroborate the hypothesis that a TH1 response capability is incompatible with implantation/nidation and that a TH2-response mode is required to accomplish this function successfully (*Yeaman* et al., 1998). Further evidence for this hypothesis is the observation that this CTL activity remains constant in post-menopausal women. However CTL activity remains constant in vagina and cervix (rat, mouse) during the entire ovarian cycle. The importance of CTL during infection is well proven by even a partial depletion *in vivo* of this T-cell population (*Parr* and *Parr*, 1998).

NATURAL KILLER CELLS (NK-CELLS)

NK cells are also randomly distributed throughout the tract in relatively large numbers. These cells are composed of three subgroups (mouse). The first group contains the characteristic LGL-1 membrane antigen and the absence of intracellular perforin. The second group is characterised by the presence of both the LGL-1 antigen and intracellular perforin. The third group lacks the membrane marker and contains large stores of perforin. The latter is the fully activated NK-cell phenotype. In the mouse the first type is present up to the 6th day of gestation and accumulates around the developing metrial glands. The second group becomes numerous by day six of gestation. Perforin producing cells become detectable by day 12 and the fully activated form by day 14 of gestation (*Parr* et al., 1991). Once established in the reproductive tract, these NK-cells seem to constitute a population that is separate from the circulating NK-cell pool. It has been suggested that NK-cells influence, like CTLs, the development of uterine gland and that the uterine stroma influences the maturation of the NKs (*Parr* et al., 1991). Thus it is plausible that NK-cells take over CTL-functions once the embryo is implanted.

ENDOMETRIAL GLAND LYMPHOID AGGREGATES

Small lymphoid aggregates are associated with the endometrial/cervical (human) glandular epithelium. The small aggregates consist of a core of B-cells surrounded by $CD8^+/CD4^-$ T-cells which in turn are surrounded by macrophage like cells. These aggregates develop during the high progesterone phase of the cycle and continue into pregnancy. Current opinion is that these aggregates have no "defence" function but act as tolerogenic centres to support the implantation of the allogeneic embryo. Unlike CTL activity these gland-

associated lymphoid aggregates are no longer present in the post-menopausal reproductive tract (*Yeaman* et al., 1997).

INTRA-EPITHELIAL LYMPHOCYTES (IELs)

IELs are very numerous in the epithelium of the reproductive tract. Similar to the intestine the larger majority have γT-Cell Receptor (TCR) (*Wira* et al., 1994). IEL numbers increase during di-oestrus (when the vaginal squamous epithelium is thinnest) followed by a decline during pro-oestrus and reaching the lowest numbers at the time of oestrus. Their exact function, like in the gastro-intestinal tract, is not well understood. The IELs are often associated with Langerhans cells in vagina and cervix and express CD4. In the cervix (human) and uterus the $CD8^+$ phenotype becomes most prevalent. It has been speculated that the IELs are influenced by interferon-γ (INF-γ) produced under high progesterone conditions by glandular and stromal epithelial cells. This is also the time of peak production of IL-6. The connection between INF-γ, IL-6 and IELs is not very well understood (*Wira* et al., 1999). Their increase during the latter part of the cycle might indicate that IELs either take over some CTL-function or have a tolerogenic function.

POLYMORPHONUCLEAR LEUKOCYTES (PMNs)

PMNs are well distributed throughout the healthy reproductive tract. However, during any form of infection they increase spectacularly and usually migrate in large quantities into the lumen of the tract. PMNs constitute a very effective and fast defence and “clean up” mechanism. Interestingly PMNs produce substantial amounts of INF-γ and seem to induce the epithelia to increase production of pIgR and the C3 component of complement by luminal epithelial cells, thus amplifying their own activity (*Givan* et al., 1997; *Yeaman* et al., 1998).

CONCLUSION

This short summary attempts to demonstrate how the individual immune components of the female genital tract interact and determine the microenvironment of the vagina. Because prophylaxis (immunisation) has a very high priority in both human and veterinary medicine, it is important to emphasise the following aspects:

1) The homeostasis of the vaginal flora seems to be maintained not by IgA, as in the intestine, but by IgG. The working hypothesis is that oestrogen induces glycogen production in the luminal layers of the vaginal squamous epithelium. When these cells slough and disintegrate the existing flora expands and at this time antigens of new strains/types can penetrate to iliac and supra-aortic lymph nodes where a preferentially IgG response is induced. This IgG accumulates during pre-oestrus under the granular layer (glycogen cells) and is released during the next cycle eliminating new or too dominating strains/types. Some authors claim that the mucosae-associated lymph-nodes such as the iliac and mesenteric nodes produce intrinsically

more IL-4 and hence tilt the outcome of the response somewhat toward an IgA response. However there is little firm evidence for this assumption and the general finding after experimental infections is that IgG constitutes indeed the major isotype of the response.

2) The reproductive tract is not the ideal place to induce a strong IgA response since no real Mucosa Associated Lymphoid Tissue (GALT) is connected with the genital tract. The epithelium of vagina, cervix and uterus seem to contain an extra capacity for the transport of IgA induced at a remote mucosal site of the upper respiratory or digestive tract , e.g. intra-nasal or rectal, (*Crowley-Norwick* et al., 1997) produces possibly sufficient IgA in the circulation to protect the surface of the reproductive tract. This has been demonstrated, e.g. by the intranasal administration of an adenovirus vector containing a glycoprotein (gB) of HSV-2 (*Galichen* and *Rosenthal*, 1996).

Consideration should also be given to the concept that a disturbance of the beneficial vaginal flora is likely to originate from pathogens residing in the digestive tract. The probability that these pathogens have previously been processed by the GALT is high ; resulting in a substantial IgA response/memory and the concomitant suppression of the IgG response. Thus in the case of invasion by a pathogen of intestinal origin there should exist a population of IgA memory cells , a circulating pool of specific IgA and likely a population of cells that suppress an IgG isotype response. The inflammation will induce mucosae specific addressins which in turn results in the homing of IgA committed cells to the reproductive mucosa (*Szabo* et al., 1997) and the large capacity of the pIgR will transport both locally produced IgA and circulating IgA into the lumen of the reproductive tract. This would explain the strong IgA response against the haemolysin of *Gardnerella vaginalis* when this pathogen infects the vagina (*Gauci*, 1999).

3) Contrary to paradigms it is not a requirement for protection of the genital tract that an IgA response is induced given the fact that IgG is the dominant isotype and antibody in vaginal secretions. The timing of antigen application, however, seems to be crucial because access to the iliac lymph nodes seems only possible towards the end of the ovarian cycle. The feasibility of this route of immunisation when the timing in the ovarian cycle is observed is proven by experiments using attenuated HSV-2 in progesterone treated rats (*Galichen* and *Rosenthal*, 1996).

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MUCOSAL IMMUNE RESPONSE AND MICROBIAL FACTORS IN BACTERIAL VAGINOSIS

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SUMMARY

Bacterial vaginosis (BV) is a polymicrobial syndrome afflicting about 20% of women in developed countries and more than 40% of African women. The synergy among different microorganisms causing the setting and the persistence of an altered vaginal flora and the role of host response are still to be understood. The better characterised microbial factors present in BV are the *Gardnerella vaginalis* haemolysin (Gvh), a pore-forming toxin which triggers a specific IgA response in about 50% of patients with BV and the sialidase activity which is detectable in 75% of women with BV. The absence of anti-Gvh IgA response in BV is associated with high levels of sialidase activity and IgA cleavage. *G. vaginalis* colonisation could be harmless until the host is able to counteract its toxin. When growth of other anaerobic bacteria occurs hydrolytic enzymes impair the local defence machinery of the host allowing Gvh and other microbial factors to elicit all their virulence. Increasing evidence points to synergistic relationships between *G. vaginalis* and anaerobic bacteria as pathologic core of BV.

INTRODUCTION

Bacterial vaginosis still represents an ecological mystery. BV is a very common cause of vaginal discharge in women of reproductive and postmenopausal age (*Eschenbach*, 1993). It is a polymicrobial syndrome characterised by an imbalance of vaginal flora, due to a marked reduction of the normal Lactobacilli replaced by large numbers of *Gardnerella vaginalis*, anaerobes including *Bacteroides*, *Prevotella*, and *Mobiluncus* species, and *Mycoplasma* (*Hill*, 1993). BV infrequently produces severe symptoms; the main complaint of women is malaodour mostly perimenstrual or postcoital. Several studies have shown that BV is implicated in serious obstetric and gynecologic conditions, such as preterm labour, preterm birth (*Gravett* et al., 1986), low birth weight (*Hillier* et al., 1995), premature rupture of membranes, histologic chorioamnionitis (*Hillier* et al., 1988), and pelvic inflammatory disease (PID) (*Soper* et al., 1994). Recent reports documented the correlation of BV with susceptibility to HIV infection (*Cohen* et al., 1995; *Sewankambo* et al., 1997).

Clinically, the diagnosis of BV is based on four factors, the so called Amsel criteria (*Amsel* et al., 1983), including the presence of a milky homogeneous white discharge, a vaginal pH>4.5, a positive amine odour test (release of a fishy amine odour when vaginal fluid is mixed with 10% KOH),

and the identification of clue cells (vaginal epithelial cells heavily coated with bacilli) seen on microscopic examination. Since the original study by *Spiegel* et al. in 1983, several other studies have evaluated the reliability of Gram-stained vaginal smears for diagnosing BV (*Mazzulli* et al., 1990; *Nugent* et al., 1991), presently the Gram-evaluation according to Nugent score is considered the gold standard (*Schwebke* et al., 1996). The Gram-stain of vaginal fluid from patients with a clinical diagnosis of bacterial vaginosis has a characteristic appearance: It shows many small Gram-negative or Gram-variable organisms resembling *G. vaginalis* (which comprise *Bacteroides*, *Prevotella*, and *Porphyromonas* spp.), curved Gram-variable rods (*Mobiluncus* spp.) in the absence of *Lactobacillus* species (large Gram-positive rods). The three morphotypes are quantitated and summed to yield a score 0-3 for normal flora, 4-6 for intermediate abnormal flora, 7-10 for BV (scores 9 and 10 are attained only if curved rods are present) (*Nugent* et al., 1991). Frequently clinicians make empirical diagnosis without the aid of microscopy and/or vaginal swab specimens are often sent to the diagnostic microbiology laboratory for culture test of *G. vaginalis*, but as small numbers of *G. vaginalis* can be detected also in healthy women, BV is frequently misdiagnosed.

Many basic questions regarding BV remain unanswered. It is not known what triggers the shift of the vaginal flora from lactobacilli colonisation to facultative and anaerobic overgrowths. About half of the patients having BV have no symptoms. One third of pregnant women spontaneously recover from BV in the late pregnancy. Although the epidemiological association between BV and preterm delivery has been well documented, it is still questioned if treatment during pregnancy prevents adverse outcomes. In the non-pregnant patients debate is still open on the influence of sexual habits, contraceptive methods, hormonal status on setting and persistence or recurrence of BV. Questions regarding treatment of BV as a means to prevent PID and sexual transmission of HIV are also still open.

For most clinicians the BV diagnosis is still doubtful and a simple, objective and inexpensive diagnostic test should be highly helpful. A reliable criterion for selecting patients who really need antibiotic treatment has not been proposed so far.

GARDNERELLA VAGINALIS CYTOLYSIN

G. vaginalis is a small, non-motile, catalase negative, pleomorphic bacillus. Although *G. vaginalis* has a Gram-positive organisation of the cell wall, it stains frequently like a Gram-negative or Gram-variable coccobacillus (*Catlin*, 1992).

Gardner and Dukes identified the bacterium in 1955 as the aetiological agent of the so-called "non-specific" vaginitis later defined bacterial vaginosis because of the absence of inflammatory signs (*Gardner* and *Dukes*, 1955). *G. vaginalis* adheres to exfoliated epithelial cells forming the so-called 'clue cells' which constitute the main diagnostic marker for BV. The role of *G. vaginalis* in BV has been long debated, in fact it is the only bacterium invariably present in high numbers in BV, but rarely it is the only microbial species colonising patients. The lack of inflammatory reaction

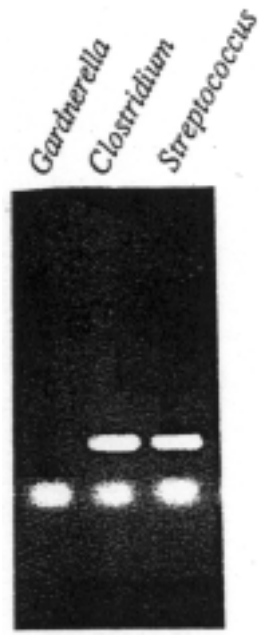

Figure 1: PCR amplification of *Gardnerella vaginalis*, *Clostridium perfringens*, *Streptococcus pyogenes* DNAs obtained with synthesised degenerated primers corresponding to conserved regions upstream and downstream the consensus undecapeptide sequence ECTGLAWEWWR.

and the detection, although in low amounts, in healthy women of *G. vaginalis* portrayed this bacterium as non-invasive and of low virulence.

Exponentially growing *G. vaginalis* releases in the culture broth a haemolysin (Gvh) which is the haemolytic factor responsible for the β-haemolysis of the bacterium on human blood agar plates. The toxin provokes a swelling of human erythrocytes which precedes haemoglobin release and ghost formation. Cell lysis occurs by osmotic unbalance due to the formation of transmembrane pores on the target plasma membrane (*Cauci* et al., 1993a). The protein is hydrophobic and requires ammonium acetate for preservation of activity. Very interestingly protein stability is essentially constant in the pH range 5-7 (pH values found in vaginal fluids of women with BV) whereas a dramatic loss of toxin activity is achieved by storing it at pH 4 (pH value of healthy women). Haemolysis occurs with a sigmoid dose dependence profile indicating that a co-operative aggregation of toxin monomers on the target membrane forms the effective pores. The lytic process exhibits a marked temperature dependence with maximal efficiency at 37°C. At variance with other pore-forming agents the best pH value for Gvh lysis is around 5 (*Cauci* et al., 1993b). So the toxin appears to elicit its maximal activity in conditions present in the vaginal niche during BV. Gvh binds avidly to lipid vesicles comprised of cholesterol and phospholipids especially if a negatively charged phospholipid as phosphatidyl serine is present. Overall features of Gvh resemble those of the cholesterol-binding toxins produced by Gram-positive bacteria belonging to the genera *Bacillus*, *Clostridium*, *Listeria*, and *Streptococcus*. These toxins form a family of antigenically related membrane-damaging proteins

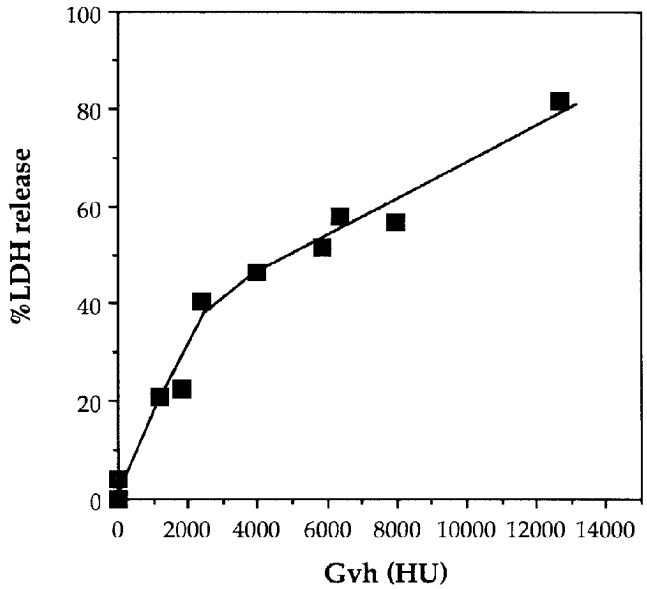

Figure 2: Release of lactate dehydrogenase (LDH) from human amnion cells in primary culture after 15 min incubation at 37°C with increasing amounts (expressed in haemolytic units, HU) of the *Gardnerella vaginalis* toxin (Gvh).

having cholesterol-anchoring properties. All these toxins (perfringolysin O (PFO), streptolysin O (SLO), listeriolysin O (LLO), alveolysin (ALV), pneumolysin (PLY)) share homologies in the protein sequence. In particular they contains a conserved undecapeptide sequence (ECTGLAWEWWR) in the C-terminal part region which is functionally important for activity (*Alouf* and *Geoffroy*, 1991). To verify if also Gvh sequence contains the conserved undecapeptide we synthesised degenerated primers corresponding to upstream and downstream conserved regions and made PCR amplification using *G. vaginalis*, or *Clostridium perfringens*, or *Streptococcus pyogenes* DNA, obtaining a good amplification in the case of *Clostridium* and *Streptococcus* but not in that of *Gardnerella* (Figure 1). This finding indicates that Gvh sequence must diverge from that of the cholesterol-binding toxins. Moreover Gvh is not antigenically related to such proteins as it is not recognised by an anti-SLO serum, nor is sulphydryl-activated as are these toxins being instead deactivated by reducing agents (*Cauci* et al., 1993a). Although in many respects Gvh action resembles PFO, a member of the cholesterol-binding, sulphydryl-activated cytolysins, the *G. vaginalis* toxin is an unique cytolysin suitable to act in the vaginal ecosystem.

Gvh could damage host cells other than erythrocytes perturbing host defence. In fact high doses of Gvh are cytolytic for human umbilical vein endothelial cells and for human leukocytes (*Rottini* et al., 1990). Gvh could be involved in the damage to the epithelial cells, which after desquamation form the clue cells.

We have measured Gvh cytotoxic effects also on amnion cells in primary culture (Figure 2) by monitoring the release of lactate dehydrogenase (LDH) after 15 min incubation at 37°C. This finding supports the hypothesis of a di-

rect role of the toxin in the intrauterine niche and in obstetric complications.

At low doses Gvh causes chemotaxis inhibition of human polymorphonuclear leukocytes *in vitro* (*Shubair* et al., 1993), so the toxin could play a role in the inhibition of inflammatory response in patients with BV in concert with other inhibitors of leukocyte functions as succinate produced by obligately anaerobic rods.

ENZYMATIC MICROBIAL FACTORS IN BACTERIAL VAGINOSIS

Extracellular mucolytic enzymes, including mucinases and sialidases have been found in vaginal fluids of patients with BV (*McGregor* et al., 1994). Mucins play a relevant role in the homeostasis of the female reproductive tract and are important in the mucosal host defence against various microorganisms as bacteria, protozoa and viruses, and cytotoxic substances. Particularly in pregnancy the mucin plug is considered to prevent the entry of microorganisms into the uterine cavity. Bacterial mucinases play complex roles in the physical defence of the host, in cell recognition and in microbial pathogenesis.

Sialidases, or neuraminidases, have been implicated in the pathogenesis of many diseases. They catalyse the removal of terminal sialic acid residues from various glycoconjugates as mucins, and play roles in bacterial nutrition, cellular interactions and immune response evasion (*Corfield*, 1992; *Pilatte* et al., 1993; *Taylor*, 1996). Bacteria involved in urogenital infections are able to produce sialidases. *Briselden* et al. (1992) demonstrated increased levels of sialidase activity in vaginal wash samples obtained from women with BV and they correlated sialidase activity mainly with the presence of high titres of *Prevotella* spp. and *Bacteroides* spp. Sialidase activity found in vaginal fluids exhibits maximal activity in the pH range from 4.5 to 5.5 (typical pH of women with BV) whereas a drastic reduction is observed at lower pHs (values found in healthy women). An average 80% reduction was measured at pH 3.5 (*Cauci* et al., 1996). This observation could be of relevance in the strategies adopted to eradicate bacterial vaginosis by local therapy.

Noteworthy sialidase activity was present in all women who had recurrent BV 1 month after oral therapy with metronidazole or ampicillin (*Briselden* et al., 1992). Persistence or recurrence of sialidase activity in vaginal fluids of pregnant women with BV treated with 2% clindamycin cream or placebo correlate with an increased risk of preterm birth and low birth weight, whereas mucinase activity did not (*McGregor* et al., 1994).

Proline aminopeptidase activity has been identified in the vaginal fluids of women with BV. Prolidase is presumed to be produced by *G. vaginalis* and *Mobiluncus* spp. Other genera known to produce prolidases include *Peptostreptococcus*, *Streptococcus*, *Actinomyces*, *Propionibacterium* (*Schoonmaker* et al., 1991). The prolidase assay shows an excellent correlation with Gram-stain diagnosis, and has been proposed as a diagnostic test for BV (*Hillier*, 1993), but unfortunately it requires many hours of incubation so it is more suitable for research purposes than as doctor office test. Additional work is needed to better identify the microorganism(s) responsible for prolidase activity and clarify the role of this enzymatic activity in BV.

Several other bacterial enzymes including phospholipases have been suggested as potential virulence determinant

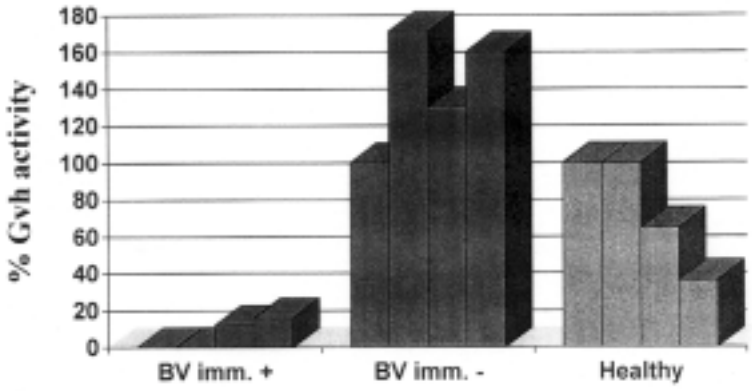

Figure 3: Haemolytic activity of Gvh after *in vitro* 1:1 incubation with vaginal washings from four patients having BV positive for anti-Gvh IgA (BV Immune+), four patients with BV and no detectable anti-Gvh IgA (BV Immune-), and four healthy women without anti-Gvh IgA.

in BV but production of such enzymes has been proved only by testing the *in vitro* release from cultured isolates. Proteases producing microorganisms include *Prevotella melaninogenica*, *Prevotella bivia*, *Bacteroides fragilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus* spp., *Ureaplasma urealyticum*.

Production of virulence factors such as sialidases, collagenases, prolidases and proteases by microorganisms implicated in BV may play key roles in the perturbation of host defences, allowing ascent of microorganisms into the upper genital tract and/or favouring subsequent microbial or viral infections (*McGregor* et al., 1986; *McGregor* et al., 1994).

ANTI-HAEMOLYSIN IgA RESPONSE AND SIALIDASE ACTIVITY

A specific IgA response against Gvh has been documented in the vaginal secretions showing the involvement of this toxin in the *in vivo* colonisation by *G. vaginalis* (*Cauci* et al., 1996) and the ability of the bacterium to activate the immune response in spite of the paucity of inflammatory signs. The purified toxin is a suitable antigen to evaluate the host response to *G. vaginalis* independently of the particular strain of the bacterium harboured by the woman. Previous attempts to evaluate the host immune response in serum of BV patients demonstrated that using the whole bacterium as antigen the immune response is detectable only if the *G. vaginalis* strain colonising the woman is employed (*Ghione* et al., 1989). Anti-Gvh IgA levels are significantly higher in vaginal fluids of women with BV than in the normal controls: anti-haemolysin antibodies are detectable in about half of the women with BV, 20% of women with intermediate microflora, and in 9% of healthy women (*Cauci* et al., 1996). Vaginal fluids of women with BV having anti-Gvh IgA (Immune+) are able to completely inactivate *in vitro* the cytolysin, whereas those of women with BV without anti-Gvh IgA (Immune-) are inactive; some of the vaginal fluids of healthy women (negative for anti-Gvh IgA) are able to partially deactivate the toxin likely through aspecific mechanisms (Figure 3). It is to

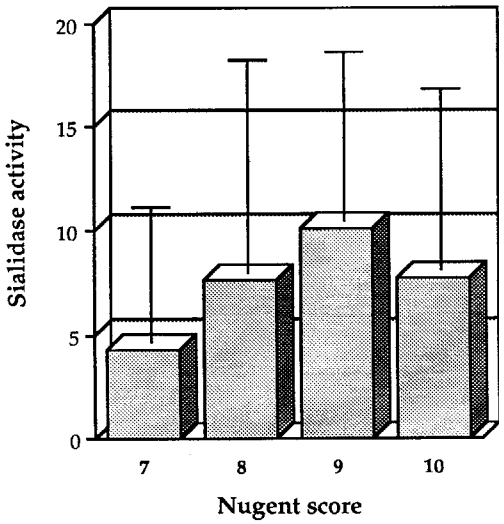

Figure 4: Average values (error bars indicate standard deviations) of sialidase activity in vaginal fluids of 57 women with BV having different Nugent scores. No subset was statistically different from each of the others (using Student t test for unpaired observations, $p>0.05$).

be underlined that the absence of an anti-Gvh response in BV patients colonised by *G. vaginalis* is not due to the inability of the strains harboured by the patients to secrete the haemolysin and that the two subgroups of BV patients (Immune+ and Immune-) are indistinguishable by the normal criteria used to diagnose BV: pH, amine odour, discharge, clue cells, Nugent score.

Sialidase activity has been reported to be present in 84% (*Briselden* et al., 1992), 45% (*McGregor* et al., 1994) and 75% (*Cauci* et al., 1998a) of the vaginal fluids of women with BV. Sialidase activity is absent in vaginal fluids of women with *Candida* vaginitis (without concurrent BV), and very low values are detectable in only 5% of healthy women. Interestingly sialidase levels of clinical diagnosed BV are not increasing with the value 9 and 10 of the Nugent score (Figure 4). This finding confirms previous *in vitro* observations on the inability of *Mobiluncus* spp. to produce sialidases (*Briselden* et al., 1992). As very few isolates if any of *G. vaginalis* secrete sialidases, anaerobic Gram-negative rods such as *Prevotella* spp., and *Bacteroides* spp. are the most probable source of sialidases in BV.

When the two subgroups of women with BV (Immune+ and -) were examined 87% of the women Immune- were positive for sialidase, whereas 59% of women Immune+ were positive. Moreover women with BV and no detectable anti-Gvh response show a five-fold higher sialidase mean value than women with BV positive for anti-Gvh response (*Cauci* et al., 1998a). This study demonstrated that high sialidase activity is associated with the absence of an IgA local immune response to *G. vaginalis* cytolysin.

Recently the existence of two main different subgroups of BV patients has been further substantiated by analysis of immunoglobulin integrity in vaginal fluids: the subgroup of patients without anti-Gvh response shows extensive degradation of IgA and IgM (*Cauci* et al., 1997; *Cauci* et al., 1998b).

SYNERGY BETWEEN *GARDNERELLA VAGINALIS* AND ANAEROBIC BACTERIA

Synergistic mechanisms among microorganisms involved in bacterial vaginosis have been already hypothesised in the past (*Pheifer* et al., 1978; *Chen* et al., 1979; *Spiegel* et al., 1980). Very recent studies support this hypothesis: a symbiotic relationship between *G. vaginalis* and *P. bivia* involving ammonia utilisation by the former has been described by *Pybus* et al. (1997); the combination of *G. vaginalis* and anaerobic bacteria and/or *M. hominis* has been proposed as pathologic core of BV on the basis of microbiologic epidemiology by *Thorsen* et al. (1998).

The only virulence factor so far characterised of *G. vaginalis* is its cytolysin, Gvh. The toxin is suitable to work in the vaginal niche but it is largely inactivated at pH 4, becoming more active at pH around 5 that can result from amine release by anaerobes.

It is conceivable that Gvh contributes to survival of *G. vaginalis* by providing metabolites, but the supply of nutrients especially of iron could also favour colonisation of strictly iron requiring species as *Bacteroides* and *Prevotella* spp.

The detection of a specific IgA response against Gvh induce to hypothesize that *G. vaginalis* colonization is relatively inoffensive until the host immune system is able to counteract the cytotoxic effects of the toxin. This is in keeping with the observation that colonization by *G. vaginalis* in patients without anaerobic overgrowth may frequently be asymptomatic and transient. When synergistic growth of anaerobic bacteria occurs, microbial factors as hydrolytic enzymes and/or exoproducts impair the immune response of the host permitting the toxin (and other virulence determinants) to elude clearance and explicate all the virulence potential.

Sialidases, mainly produced by anaerobes, may promote virulence not only by destroying the mucins and enhancing adhesion of bacteria but also by impairing the specific IgA immune response against virulence factors as cytolysins. Cleaving the sialic acid residues off the IgA molecules, makes them more accessible to protease degradation (*Kilian* and *Russel*, 1994; *Mattu* et al., 1998). Other mechanisms as the modification of the carbohydrate complexes involved in cellular recognition events and in aspecific antibacterial functions of S-IgA may be compromised. Sialidases are often one of several virulence factors secreted by anaerobic bacteria which are able to produce an array of proteases.

Bacterial proteases (as prolidases) have been detected in cervical secretions of BV patients but until now their pathologic role is still hypothetical. Our data on the *in vivo* cleavage of mucosal IgA and IgM give insights on the failure of the host to counteract bacterial proteases. In fact IgAs are considered intrinsically resistant to proteolysis and the secretory component of mucosal immunoglobulins enhances this resistance. Moreover the release of protease inhibitors in human secretions and the production of a specific immune response against bacterial proteases, should protect immunoglobulins from *in vivo* degradation. Our study, to our knowledge, is the first demonstration of the *in vivo* IgA and IgM degradation in human mucosal secretions as a consequence of microbial pathogens colonisation (*Cauci* et al., 1998b). The immunoglobulin degradation pattern, which includes several low molecular weight bands and the involvement of IgM in the degradation exclude that the cleavage is entirely due to known pro-

teases, as IgA1 proteases (*Mulks* and *Shoberg*, 1994). It remains to identify which microorganism(s) produces the proteolytic enzymes responsible for the extensive cleavage of the vaginal secretory immunoglobulins.

It has to be reminded that IgA dimers are thought to have a pivotal role in the protection of the vaginal tract. A very recent paper has shown that vaginal IgAs but not IgGs together with systemic cell-mediated immunity have a main role in the protection from viral transmission (*Mazzoli* et al., 1997). Thus the development of any HIV vaccine that should enhance the host protective mucosal immunity against penetration of viruses must take into account that a very common women disease such as BV can compromise the immunoglobulins integrity. If future studies will demonstrate that the patients with compromised local immune response show a higher risk of prematurity, bacterial invasion of the amniotic cavity, upper genital tract infections, bacterial vaginosis recurrence, susceptibility to HPV or HIV infection, sialidase activity (or others hydrolytic enzymatic activities) could become a valuable diagnostic marker for predicting the severity of the disease and helping in the choice of the chemotherapeutic treatment.

CONCLUSIONS

Much remains to be learned about the interactions among members of the vaginal microflora and factors of the human mucosal defence. Bacterial inhabitants of ecological "niches" in the female genital tract are dynamic and can vary in number and composition also in the same host. Complex interrelationships among invading microorganisms themselves and between microbial virulence determinants and host defence factors are increasingly evidenced. In particular the role of hydrogen peroxide producing Lactobacilli present in healthy women is under active investigation. Some synergistic mechanisms in which *G. vaginalis* is implied have been clarified increasing our understanding of BV pathogenesis. More extensive studies of the mechanisms involved in the homeostasis of the vaginal ecosystem should indicate new avenues for prevention of preterm labour, low birth weight, transmission of STD and susceptibility to sexual infection by HIV.

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VAGINAL MICROECOLOGY AND VULVAL DISCHARGE IN SWINE

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SUMMARY

This article reviews current knowledge concerning the occurrence of vulval discharges in sows. Both the role of the vaginal flora and the management practices on the herd are emphasised. It appeared that the vaginal flora changes with the reproductive stage of the sow, and that many of the bacteria of the vaginal flora are similar to those frequently reported in clinical cases of the vulval discharge syndrome. Factors that contribute to a high contamination of the urogenital tract of sows, such as low hygiene standards and poor housing conditions are important risk factors for the disease. Poor management of the boar and insufficient oestrus detection also increase the risk of infection. The cyclic variation of uterine defence capacities throughout the reproductive cycle plays an important role in the pathogenesis of vulval discharge. Defence mechanisms decrease towards the end of oestrus and at onset of metoestrus i.e. when oestrogen levels have become low and when progesterone levels increase. Criteria that can be used to discriminate between physiological and pathological discharge, and that permit to pinpoint the exact infection site in case of a pathological discharge, are discussed. There is no uniform treatment and the results of antimicrobial therapy are often disappointing. Prevention rather than treatment should be encouraged. Preventive measures are largely based on minimising or eliminating the different risk factors for the disease.

INTRODUCTION

Infections of the urogenital tract in sows are commonplace in many modern swine facilities. Although the infection can affect one or more organs of either the reproductive or urinary tract, the most common clinical sign associated with these infections is the appearance of a vulval discharge. This article will review conditions that influence the occurrence of the vulval discharge syndrome. Although the vaginal flora in sows is not documented that much in veterinary medicine as it received attention in human medicine (*Spiegel*, 1991; *Sobel*, 1998), its role in the occurrence and prevention of vulval discharges will be emphasised. The role of the boar and management practices will also be discussed.

ANATOMICAL CHARACTERISTICS OF THE SOW'S REPRODUCTIVE TRACT

The vulva of the sow has two fleshy lips that contain many blood vessels. The vagina is approximately 400 mm long, and consists of two parts: the vestibula (approximately 70 mm), situated caudally from the opening of the urethra, and the fornix vaginae situated between the opening of the urethra and the cervix. The cervix is approximately 100 mm long. Tortuous cartilaginous folds form a corkscrew cervical canal that can be passed only at farrowing and during oestrus. The folds are less prominent to the uterine side. The uterus or womb consists of a short body (50 mm) and two horns. The length of the horns is approximately 500 mm in non-pregnant gilts, but the horns can reach 1-1.8 m during pregnancy. The junction from the uterus to the oviduct is not sharply delineated. The oviduct is 40-80 mm long. The ovaries are 10-20 mm and are embedded in a great bursa ovarica.

THE VAGINAL FLORA

The flora of the vagina of healthy sows consists of a wide range of bacteria, including aerobic and anaerobic species. The most representative are *Streptococcus* spp., *Staphylococcus* spp., Enterobacteria, *Corynebacterium* spp., *Micrococcus* spp. and *Actinobacillus* spp. Many of these bacteria are similar to those frequently reported in clinical cases of the vulval discharge syndrome (*De Winter* et al., 1995). The number of bacteria decreases steadily from the caudal to the cranial vagina (*Berner*, 1984). *Bara* et al. (1993) demonstrated in a longitudinal study that the cervical-vaginal microflora changes continuously due to intrinsic mechanisms of the sow's reproductive tract, such as cyclic hormonal pattern, secretion of immunoglobulins and mucus, and the phagocytic activity of the granulocytes. The highest isolation rates were obtained on the day of farrowing and the lowest 3 weeks after mating. The second highest number of positive samples was found immediately after mating. Although there was no significant difference among sows of different parities, there was a trend for older sows to have more positive samples after farrowing. Fifty-five percent of the cultures were pure, 45% were mixed. *McLean* and *Thomas* (1974) documented that parturition and a dietary change resulted in an upgrowth of *E. coli* and other bacteria in the reproductive tract of sows. *Staphylococcus hyicus*, the aetiological agent of exudative dermatitis in piglets, is also part of vaginal flora of the sow (*Elliott*, 1986). *Wegener* and *Skov-Jensen* (1992) showed that *Staphylococcus hyicus* strains are transferred from the vagina of sows to the piglet at birth, and that these bacteria become part of a stable skin flora of the offspring.

Several investigators isolated bacteria from the uteri of normal sows. *Scoffield* et al. (1974) and *Ludwig-Stössel* (1985) isolated bacteria in the uterus in about 50% of the sows they examined at slaughter. They isolated mainly *E. coli*, *Staphylococcus* spp. and *Streptococcus* spp., in some cases *Arcanobacterium pyogenes*, *Enterococcus* spp. and *Pasteurella multocida*. However, positive isolation rates in uteri from slaughtered sows should be interpreted with great caution, because breeding pigs are often culled due to reproductive failure

and uteri from slaughtered sows may be contaminated by urine passing retrograde via the vagina through the cervix. *Meredith* (1986) reported that a normal uterus must be sterile. He only isolated bacteria from the uterus within a few days after parturition or during the first day after service. The degree in which the presence of bacteria in the uterus of normal sows influences early pregnancy is poorly understood. *Scoffield* et al. (1974) showed that pregnancy can be established in an infected uterus, but that embryonic survival rates are much lower than in a sterile uterus.

THE VULVAL DISCHARGE SYNDROME

The potential areas from which vulval discharges can arise include the vulva, the vagina, the cervix and the uterus. Discharges can also arise from infection of the kidneys (pyelonephritis) or the bladder (cystitis) with pus being passed in the urine. In this article, emphasis will be placed on infections of the genital tract. Vaginal infections mostly occur concomitant with chronic infections of the uterus or with chronic urinary tract infections. Occasionally, some sows and virgin gilts with vulval discharge have obvious vaginitis and cervicitis, with no or only minimal changes in their uteri. This finding suggest either that an ascending infection of the reproductive tract has not yet reached the uterus, or that a uterine inflammation has resolved. The spontaneous appearance of the syndrome in many unrelated herds supports the view that the infection arises from the existing bacterial population in the herd, rather than from the introduction of a specific organism. *De Winter* et al. (1995; 1996) were able to reproduce the syndrome vulval discharge experimentally by inoculation of *E. Coli* or *Staphylococcus hyicus* strains into the uterus. In one study, *Streptococcus suis* type II has been shown to be the cause of an outbreak of vaginitis in gilts (*Sanford* et al., 1982). Evidence is lacking that viral infections play an important role in the vulval discharge syndrome.

EPIDEMIOLOGICAL ASPECTS

Vulval discharges are often seen in sows during the first and second oestrus after weaning, after farrowing and in gilts, but they are rare in immature gilts, lactating sows, and in pregnant sows. Some epidemiological factors that should be considered in the syndrome are shortly discussed here.

Hygienic measures

Poor hygienic measures in the farrowing, gestation and mating units lead to an accumulation of wastes, and to an increased risk of vulval discharge. When units are continuously used without cleansing, the vulvovestibular tissues of the sows are dirty and highly contaminated (*Muirhead*, 1986; *Heard*, 1986). Poor hygiene in the boar units also increases the risk of infection. Strict hygiene is also required during intervention at farrowing and during mating or artificial insemination (AI) procedures.

The role of the boar and AI

The male reproductive tract and especially the preputial diverticulum of the boar contains many bacteria similar to those found in the sow's reproductive tract. Many of these bacteria may be transmitted from boar to sow during

coitus. The risk for infection of the sow is much higher when the content of the preputial diverticulum is manually squeezed into the vagina at service. Remating discharging sows, multiple matings and the use of cross-serving one sow with a number of boars provide a potential risk for the spread of venereally related infections. Trauma to the female urogenital tract at breeding facilitates colonisation by facultative pathogenic bacteria. Trauma occasionally occurs when the size of the boar and the sow is quite different. If a boar has very long back legs, the penis enters the vagina in a downward movement and the tip of the penis may enter and damage the urethral orifice.

Infection of the sow's reproductive tract may also occur during AI. Fresh semen from fertile boars used in different AI centres contained 10^2-10^5 bacteria per ml (*Weitze* and *Rath*, 1987). Although contamination of diluted sperm used for insemination cannot be ruled out, it is probably of lesser importance because of addition of antibiotics. Only in case of antibiotic resistance, the diluted sperm can be a source of infection. However, infection of the uterus during AI is mainly due to unhygienic procedures and to incorrect time of insemination. The latter is very important under practical circumstances and is mainly due to insufficient oestrus detection.

Housing conditions

Urogenital infections occur more frequently in sows housed in crates and in tethered sows compared to sows grouped in stalls (*Busse* et al., 1982). Discharges in virgin gilts are often seen within a few days after addition to the breeding herd, and usually after initial boar exposure to initiate oestrus cyclicity. Housing these gilts in stalls facilitates the spread among penmates.

Under intensive confinement conditions, the vulva of sows is often placed in direct contact with faeces for long periods, both in farrowing and dry sow accommodations (*Smith*, 1983). When the construction lacks a good drainage of faeces, there is a build up of faeces behind the sow at the bottom of the tailgate. Insufficient ventilation rates can lead to high relative humidity levels, and consequently to a higher bacterial contamination of the units and the animals. Sows housed in crates and tethered sows often have a lack of exercise. This causes less frequent urination and predisposes to urogenital infection, especially to infection of the urinary tract (*Madec*, 1984).

General health of the sow

Sows with leg-weakness will assume a dog-sitting position which helps to force faecal material into the vagina. Sows with urinary tract disease are more susceptible to ascending infections of the vagina, cervix and uterus (*von Both* et al., 1980; *Moller* et al., 1981; *Berner*, 1984). Prolonged farrowings, retention of remnants of the placenta, and dead piglets lead to more contamination of the genital tract. Congenital defects of the urogenital tract or trauma during parturition e.g. incomplete closure of the vulva and erosion of the thick folds of the cervix in old sows, facilitate colonisation of possible pathogenic bacteria. Apart from an infectious cause, vulvovaginitis may be caused also by oestrogenic mycotoxicosis (*Campbell*, 1988).

Other management practices

An inadequate replacement policy of the breeding stock leads to a higher incidence of vulval discharge. An imbalance in the age structure of the sows appears if there are many gilts (start-up herds and herds with a high turn-over) or many old sows (>30% of females ≥ fifth parity) in the herds. Vulval discharge problems can be more severe and more prevalent in herds with a short lactation length (<21 days).

PATHOGENESIS

The presence of bacteria in the uterus does not always result in endometritis. Except for overwhelming infections, bacteria that enter the uterus at coitus or parturition are eliminated within a few days (*Vandeplassche* et al., 1960). The hormonal status of the sow, however, plays an important role in the elimination of uterine bacteria (*De Winter* et al., 1992). During pro-oestrus and at the beginning of oestrus, the sow has high blood oestrogen and low progesterone levels (*Dial* and *Britt*, 1986). Uteri of pigs and other animal species are better able to eliminate infections caused by intrauterine deposition of either contaminated semen or an inoculum of one of several bacteria while under oestrogen domination than when under progesterone influence (*Meredith*, 1986; *De Winter* et al., 1994). Experimental infections conducted by *De Winter* et al. (1996) showed that vulval discharge occurred only in sows with blood progesterone levels exceeding 3.00 ng/ml. It appeared from studies in other animal species (*Roth* et al., 1982) that the higher resistance to infection during oestrus is mainly due to the influence of oestrogen. Higher oestrogen levels enhance migration of leukocytes into the uterus, and increase uterine blood flow and vascular permeability.

The secretory immune response in pigs to uterine pathogens is also influenced by ovarian cyclicity (*Hussein* et al., 1983). The number of plasma cells in different parts of the female reproductive tract appeared to be higher during oestrus than during di-oestrus. Studies in other animal species demonstrated that elevations of immunoglobulins during the follicular phase of the cycle, block the attachment sites of bacteria, agglutinate bacteria, and opsonise bacteria for subsequent phagocytosis (*Parr* and *Parr*, 1985; *Watson*, 1985). In addition to facilitory effects on uterine immunity, elevated oestrogen concentrations may promote evacuation of septic uterine secretions by stimulating uterine contractions and by maintaining cervical patency.

Especially in herds where the sows are served by AI, it is possible to inseminate at any time. In cases where oestrus detection is not carried out properly, sows may be inseminated after the end of standing oestrus. Such sows are more susceptible to uterine infections because at the end of oestrus, blood oestrogen concentrations are already low, while blood progesterone concentrations are already at 10 ng/ml (*Dial* and *Britt*, 1986; *Meredith*, 1986). Much knowledge about relationship between hormonal status and uterine defence has been obtained in studies conducted in different animal species. It merits further studies in sows specifically to confirm many of the aforementioned mechanisms.

CLINICAL SIGNS

Discharges resulting from urogenital tract infections may appear as dried deposits around the vulva and perineum. More often, they are observed as a pool on the floor underneath affected sows (*Dial* and *MacLachan*, 1988a). In some cases, it is necessary to separate the labia manually or to use a speculum in order to visualise the discharged exudate. Vulval discharges may result from either physiological or pathological conditions.

Physiological discharge

The periods in which it is quite normal for healthy sows to show evidence

of a slight discharge are: at pro-oestrus and oestrus, after service or insemination, during pregnancy and after parturition (*Muirhead*, 1986). During pro-oestrus, there is a small discharge of watery or slightly tacky mucus that can be clear or turbid. This discharge lasts until the first part of standing oestrus. The first hours after service or insemination, a discharge, probably consisting of seminal plasma is observed. The discharge observed 8 to 48 hours after service is of low volume and has a white, grey or yellow colour. It is probably caused by a reaction of the vagina and uterus to seminal debris (*Meredith*, 1982). A low volume of white to white-greyish discharge of thick mucus may be observed during pregnancy. It is generally believed that this discharge originates from the cervix and that it is caused by reaction to ascending infections (*Meredith*, 1982). Since histological processes occurring in the endometrium after parturition and during endometritis resemble each other, it is not always easy to pinpoint whether the puerperium takes a physiological or a pathological course. During a normal puerperium, lochia are expelled up to 5 days post-partum. Malodorous and excessive amounts of lochia should be considered as abnormal.

Pathological discharge

Discharges observed during other stages of the reproductive cycle cannot be considered as physiological. Clinical signs associated with pathological discharge depend upon the infection site(s). Parameters used to investigate the exact infection site include: amount of discharge, characteristics and frequency of discharge, age at which discharge occurs, presence of fertility failure, and general health status (*Dial* and *MacLachlan*, 1988a). Vaginal infections are characterised by a moderate amount of purulent discharge that occurs independent of the reproductive cycle. Generally, they are not associated with reduced fertility or systemic clinical signs. Vaginal infections are more common in gilts, and may appear in individual gilts or as epizootics. Uterine infections, except for puerperial infections, are associated with copious amounts of discharge. The discharge does not contain mucus; it is usually purulent or occasionally haemorrhage. It is commonly seen during pro-oestrus and oestrus. Uterine infections cause a transient or persistent infertility, may affect the general health of the sows, and are more common in higher-parity sows and in gilts. Postcoital endometritis typically does not produce discharge until 14 to 21 days after mating. The discharge of sows with cervicitis is small in volume, usually purulent, and not associated with the oestrous cycle. Infections of the cervix may result in reduced fertility but they are not associated with systemic signs. All parities may be affected and cervicitis often occurs concomitant with endometritis or vaginitis. A modest amount of discharge is seen with infections of the lower urinary tract. The discharge is usually purulent and/or mucoid, but it can also be mucohaemorrhagic. Occasionally, the discharge is more chalky. Urinary tract infections occur independent of the reproductive stage, and initially they do not affect fertility. There is dysuria and the general health status of the sows may be affected. Urinary tract infections can appear in all parities but they typically occur in older sows.

DIAGNOSIS

The diagnosis should start with a complete anamnesis and a study of the farm records. In many cases the obtained information permits to evaluate the duration of the problem and its possible relationship with reproductive performance results. A thorough examination of clinical signs is time-consuming but it may provide very useful information. Vulval discharges may be missed easily because the volume is often mixed up with dirt and faeces, and it can disappear quickly through the slats of the floor. Microscopical examination of discharge for presence of crystals, amorphous material or leukocytes permits to differentiate between urolithiasis and discharge resulting from inflammation. Bacteriological examination of discharged material from the vulval lips has no diagnostic value because it is always contaminated (*Meredith*, 1986).

Vaginoscopy is very useful to ascertain the site of origin of discharge. A speculum of 2 cm external diameter and a length of 30 cm allows visualisation of the vestibule, vagina and cervix. A longer speculum (40 cm) may be required in older sows to investigate the cranial part of the vagina. A more narrow speculum is advisable for gilts. Attention should be paid to the urethral orifice, sinus vaginalis, vaginal mucosa, and external os of the cervix. Increased vascularity or erosions of the vaginal epithelium, and accumulations of purulent exudate around the cervix or urethra may be present in affected animals.

Swabs for bacteriological examination can be taken from the vagina and cervix. Swab samples should be collected by rigorous rubbing of the surface of the mucosa with a sterile cotton swab inserted through and beyond the speculum. Contact with the surface skin and external vestibule should be avoided upon introduction and withdrawal of the swab and speculum. Unless the cervix is patent, as it is at oestrus, it is not possible to take uterine swabs through the cervical canal. However, isolates from the cervix relate well to those obtained from the uterus (*Brummelman*, 1980). Swabs from different sows may elicit the predominating organisms in the herd, and permit to carry out antibiotic sensitivity tests. Swabs from individual sows are worth examining but the results may not be representative for the herd. Further studies on the microbiology of healthy and diseased reproductive tracts are necessary to permit a more useful interpretation of isolation results.

Examination of the reproductive tract of sows at slaughter may be very useful to detect possible gross and histopathological lesions and to take samples for microbial culture. Inspection at slaughter, however, has some limitations that should be considered in the interpretation of the results. First, a representative number of sows should be investigated from an infected herd. This condition is not easily fulfilled since sows are often culled in small numbers, and since not every affected sow is sent to slaughter instantly. Second, affected sows should be slaughtered within two days after onset of the first observed discharge. *Muirhead* (1986) slaughtered 47 sows two to four days after occurrence of vaginal discharge, and surprisingly, there was a total lack of lesions in the urogenital tract in 21 of these sows. Third, the possibility exists that uteri of slaughtered sows are contaminated with urine that passes retrograde via the vagina through the cervix. Hence, it is advisable to complete microbial cultures with results of histopathological examinations (*Meredith*, 1986). However, the interpretation of endometrial biopsies is not easily established and requires some experience to do properly because the number of inflammation cells in the endometrium depends upon the cycle stage

(*De Winter* et al., 1995). Finally, slaughter inspection measures possible lesions at one particular point in time. No information is obtained with respect to onset and duration of infection. However, such epidemiological data may be imperative to effectively solve the problem at herd level.

TREATMENT

Treatment of urogenital infections of sows is not that much documented as it is for cows and mares. Vaginitis in sows is normally self-curing. The possibility exists, however, that the infection ascends and results in a cervicitis and endometritis (*Muirhead*, 1986). Because the corpora lutea of swine are non-responsive to prostaglandins until after day 11 to 12 of the oestrous cycle (*Conner* et al., 1976; *Guthrie* and *Polge*, 1976), it is not useful to shorten di-oestrus or to decrease the inter-oestrus interval for treatment of porcine uterine infections.

Sows with vulval discharges can be treated with antimicrobials by intrauterine, parenteral and oral routes (*Dial* and *MacLachlan*, 1988b). Drugs with potential efficacy in the therapy or prophylaxis of urogenital diseases of swine include tetracyclines, (potentiated) sulphonamides, penicillins, aminoglycosides, and nitrofurans. The choice of the antibiotic should be determined by antimicrobial sensitivity testing after the isolation of one of the more commonly observed pathogens. *Busse* et al. (1982) found that many bacteria involved in urinary tract infections show resistance to different antimicrobials. In addition, a potentially effective antimicrobial should be delivered to either the urinary tract or the uterus in concentrations that exceed the minimum inhibitory concentration (MIC) of the pathogen. The mode of administration for antimicrobials depends upon the reproductive stage of the sow or gilt. Intrauterine therapy is not always applicable because of the complex cervix. It is feasible in recently farrowed sows before cervical closure, and in the majority of dry sows and gilts during pro-oestrus and oestrus. It is seldom possible to apply intrauterine therapy in lactating sows or in cyclic sows during di-oestrus. Although the local application of antibiotics in the uterine lumen is beneficial especially in the acute stages of disease, it has also some drawbacks. The volume of infusate may be inadequate for optimum distribution throughout the genital tract, the antimicrobial may be present for an insufficient time, the therapy is time consuming, requires some experience to do properly and may introduce additional pathogens into the uterus. Thus, the oral or parenteral routes should be used for treatment of dry sows and gilts, while all three treatment routes can be used in recently farrowed sows and females in oestrus.

Muirhead (1983; 1986) reported that treatment of sows with vulval discharge due to endometritis is not very useful because subsequent farrowing rates are low. Therefore, he advised to cull all affected non-pregnant sows. All remaining sows should be injected with a long acting oxytetracycline preparation at weaning, and additionally, they should be treated by in-feed medication with 300 ppm oxytetracycline for 3 weeks. The prepuce of the boars should be injected daily with long acting oxytetracycline intramammary tubes for 5 days. These treatment regimens should be repeated every three months on at least four occasions. Treatment of the boars appeared to be essential, because discharges returned in more than 50% of the herds when treatment of the boars was neglected. *Walton* (1984) also

showed that the number of sows with vulval discharge decreased significantly when the boar's prepuces were treated every three months. According to *Plonait* (1988), intrauterine infusions with lotagen or lugol are effective. Lotagen or lugol solutions should be infused 18 hours before or 24 hours after insemination. These solutions cause a mild necrosis of the endometrium, and allow a quick regeneration within 24 hours.

In conclusion, the literature concerning treatment of urogenital infections of swine does not proclaim one standard method. Most authors agree that clinical responses to treatment are inconsistent, and that the choice of antimicrobial should follow a sensitivity testing. Although antibiotics may be useful to suppress the infection temporarily, preventive rather than therapeutic measures should be emphasised.

PREVENTION

Preventive measures are mainly based on management practices that minimise or eliminate the aforementioned risk factors for occurrence of vulval discharge (*Muirhead* and *Alexander*, 1998). Good sanitation of the units may prevent heavy faecal contamination of the vulvo-vestibular tissues. Cleansing, disinfection, and a stand-empty period of the pens are beneficial. However, these measures require that an all-in/all-out policy is adopted. Farmers should institute a routine to pursue high hygienic standards during manual interventions at farrowing, mating or insemination. Dirt on the vulva must be removed before service and boars must be assisted to minimise contamination of the penis, and to prevent injuries of the sow. In case of AI, semen collection, dilution, storage, and insemination should be performed under strict hygienic circumstances.

Sows may only be mated when they are totally receptive for the boar. Only one boar may be used to one sow, and old boars may not be used to young females, and vice versa. The identification of carrier boars in a herd may decrease coital transfer of pathogens. A policy not to mate discharging sows should be adopted. In case of AI, it is very important not to inseminate towards the end of the oestrus period. Sows may only be inseminated when there is a standing reflex for the inseminator in the absence of a boar. Consequently, it is imperative to perform a correct oestrus detection. Because oestrus detection rarely is conducted more than once daily on most commercial farms, it is usually not possible to obtain sufficiently accurate estimates of the time of ovulation. Multiple inseminations increase the likelihood that one or more breedings occur near the time of optimal fertility, but they also increase the likelihood that the last insemination takes place too late i.e. when oestrogen levels have dropped already and when progesterone levels are high (*De Winter* et al., 1996). Transrectal ultrasonic echography of the ovaries can be used to monitor the time of ovulation (*de Koning*, 1991). Optimal fertility occurs when insemination takes place within 24 hours before ovulation (*Waberski* et al., 1994; *Kemp* and *Soede*, 1996) i.e. 20-36 hours after the onset of oestrus.

Optimal prophylaxis is achieved by designing farrowing, breeding and gestation accommodations in such a manner that the sow is prevented from lying down in her own excreta. Wet pens for the sows and the boar can be avoided by a good drainage of urine and faeces, and by an optimal temperature and ventilation rate in the units. Ensurance of ade-

quate water intake and minimisation of the sedentary behaviour of sows may prevent infection of the lower urinary tract. Sows with locomotor disorders should be treated properly or they should be culled.

The culling of repeat-breeding, discharging sows decreases the likelihood of horizontal transfer of pathogens and minimises environmental contamination. In addition, culling of chronically affected sows may allow improvements in conception rates, facility utilisation, and consequently economic returns. Purchase of breeding stock from herds without history of urogenital disease should be encouraged. A balanced age-structure without too many gilts and without too many old sows should be pursued. In some herds, extending the lactation period to more than 21 days appeared to be beneficial.

Prophylactic treatment of all sows and boars is occasionally practised in an attempt to reduce the infection pressure on the farm, and to support the aforementioned routines. However, antimicrobial treatments are usually disappointing and they should not be recommended in most cases of vulval discharge.

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VAGINAL MICROECOLOGY AND THE PATHOGENESIS OF URINARY TRACT INFECTIONS

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SUMMARY

Vaginal colonisation with *E. coli* appears to be an important prerequisite for the development of UTI. Whereas colonisation with P fimbriated strains has been clearly shown to predispose to pyelonephritis, virulence factors which predispose to cystitis have not been so clearly identified. Several host genetic and behavioural factors have been found to be associated with increased colonisation with *E. coli* and other uropathogens and subsequent UTI. Such factors include having the blood group antigen nonsecretor phenotype which is associated with increased *E. coli* vaginal colonisation, using spermicidal contraceptive products which increase *E. coli* vaginal colonisation probably through adverse effects on lactobacilli, exposure to certain antimicrobials which facilitate coliform colonisation through adverse effects on the anaerobic flora, and use of oestrogen products which appear to enhance *E. coli* adherence to vaginal and uroepithelial cells.

Increased knowledge about these and other factors which influence the vaginal microecology is important if we are to develop safe and effective strategies to prevent UTI. For example, we can expect a reduction in the risk of vaginal colonisation with uropathogens and subsequent UTI by a decrease in the use of spermicide-containing products and use when appropriate of antimicrobials that have less impact on the anaerobic rectal and vaginal flora, such as trimethoprim, trimethoprim-sulfamethoxazole, nitrofurantoin, or fluoroquinolones. In postmenopausal women, topical oestrogens clearly help normalise the flora and reduce the risk of recurrent UTIs. Further research is needed to evaluate the feasibility and effectiveness of re-establishing vaginal colonisation with lactobacilli, especially H_2O_2-producing strains, and whether recolonization can reduce the risk of UTI. In addition, a better understanding of the vaginal microecology and immunity is necessary in order develop a safe and effective vaccine to prevent UTI.

EPIDEMIOLOGY

Acute uncomplicated urinary tract infections are among the most common conditions causing individuals to seek medical care. Population based studies in Sweden have demonstrated that during the first year of life, approximately 1% of boys and girls have symptomatic UTI, and that by the age of seven al-

most 8% of girls and 2% of boys have had a culture-documented symptomatic UTI (*Hansson* et al., 1997). Symptomatic infections in the first year of life are relatively more likely to be pyelonephritis whereas infections in older children are more likely to be cystitis. In the United States, it is estimated from surveys of office practices, hospital-based clinics and emergency departments that there are over eight million episodes of urinary tract infection and over 350,000 episodes of acute pyelonephritis annually (S.M. Schappert, personal communication). In a recent large prospective study of young sexually active women, the incidence of cystitis was approximately 0.5 per person-year, suggesting that the incidence of UTI may be much higher than these national estimates (*Hooton* et al., 1996a). Recurrent UTI occurs in 27% to 44% of healthy women even though they generally have anatomically normal urinary tracts (*Hooton* and *Stamm*, 1997). Urinary tract infections in young healthy men are very uncommon. Urinary tract infections in healthy postmenopausal women are probably less common than in premenopausal women, but incidence data are lacking.

PATHOGENESIS

Urinary tract infections (UTIs) in women develop when uropathogens, almost always from the faecal flora, colonise the vagina, ascend into the bladder and, in some cases the kidney. Vaginal acquisition of uropathogens from a woman's male sex partner has been reported but probably only rarely predisposes to a UTI. Most uncomplicated UTIs in women cannot be explained by underlying functional or anatomic abnormalities of the urinary tract. The initial pathogenic event in the urinary tract occurs when the bacteria attach to the mucosa by interactions between bacterial surface adhesins and complementary epithelial cell receptors, and stimulate cytokine release resulting in an inflammatory response and symptoms (*Hooton* and *Stamm*, 1996). Vaginal colonisation is a prerequisite to bladder infection, and factors which increase the risk of UTI generally do so at least in part by facilitating vaginal colonisation. Vaginal colonisation and infection are facilitated by host behavioural factors such as spermicide use and sexual intercourse (*Hooton* et al., 1996a) and genetic factors such as blood group antigen nonsecretor status (*Hooton* and *Stamm*, 1996) which are discussed below.

Certain bacterial virulence factors provide a selective advantage to those strains possessing them with regard to colonisation and infection (*Johnson*, 1991). Whether vaginal colonisation and subsequent UTI occur is the result of a dynamic interaction between host characteristics and uropathogen virulence determinants. Colonisation with P-fimbriated strains of *E. coli* is a strong risk factor for acute uncomplicated pyelonephritis. The pathogenesis of cystitis is less well understood compared with that of pyelonephritis, and there are no bacterial properties that identify "cystitogenic" *E. coli* clones or distinguish them from strains that cause acute pyelonephritis, although haemolysin, type 1 fimbriae and the prsGJ96 type of P fimbriae may occur more often in acute cystitis strains than in other *E. coli* strains (*Svanborg* and *Godaly*, 1997). The relative importance of bacterial virulence factors and host factors in the pathogenesis of most episodes of acute uncomplicated UTI is not known.

VAGINAL MICROECOLOGY

The microflora of the healthy vagina includes a large number of aerobic, facultative anaerobic, and obligate anaerobic species (*Hooton* and *Stamm*, 1996). Facultative members of the genus *Lactobacillus* are the most prevalent organisms isolated and are found in 50 to 90% of women in mean quantities of $10^{7.2}$ to $10^{8.7}$ colony forming units per gram. Obligate anaerobic lactobacilli are found in up to 60% of women in similar quantities. Aerobic Gram-positive cocci, including *Staphylococcus* species, *Streptococcus* species, and *Enterococcus* species are found in approximately 30 to 50% of women but in lower quantities compared with lactobacilli. Anaerobes outnumber aerobes overall by 10:1 (*Hooton* and *Stamm*, 1996). Alterations in vaginal microflora are thought to play a critical role in facilitating vaginal colonisation with coliforms and, thus, UTI.

It has been hypothesised but not directly demonstrated that lactobacilli protect the vagina by competitive exclusion of pathogenic bacteria (*Redondo-Lopez* et al., 1990). Hydrogen peroxide (H_2O_2), produced by some vaginal lactobacilli strains in almost all normal women, may be important in colonisation resistance (*Eschenbach* et al., 1989). For example, the presence of H_2O_2-positive lactobacilli in the vagina of pregnant women is inversely correlated with infections such as bacterial vaginosis and symptomatic candidiasis or vaginal colonisation by some genital pathogens such as *Gardnerella vaginalis*, *Bacteroides*, *Peptostreptococcus*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, viridans streptococci, and *Enterococcus* (*Hillier* et al., 1992). In addition, *Hawes* et al. (1996) demonstrated that women with H_2O_2-producing lactobacilli colonising the vagina are at less risk of acquiring bacterial vaginosis compared with women who have an absence of H_2O_2-positive lactobacilli. Vaginal lactobacilli, especially H_2O_2-producing strains, may also have a role in protecting the vagina from colonisation with uropathogens as discussed below.

E. coli frequently colonises the vagina, even in women without frequent UTI recurrences (*Hooton* and *Stamm*, 1996). However, women with a history of recurrent UTI are more likely to be vaginally colonised with uropathogens compared with those without such a history (*Hooton* and *Stamm*, 1996). Approximately 32% of prepubertal girls, 16% of pregnant women, and 41% of post-menopausal women have been found to harbour vaginal *E. coli* in prevalence surveys. An even higher proportion of women are found to be colonised when serial vaginal cultures are done. Generally, the factors that predispose to vaginal colonisation also predispose to bladder colonisation and infection. Vaginal colonisation with uropathogens, however, does not inevitably lead to UTI, and it remains to be determined why vaginal colonisation progresses to UTI in some women and not in others. It is likely that vaginal colonisation is usually a necessary predeterminant to UTI, but that other events, such as sexual intercourse, generally must occur to allow infection to occur. Published literature suggests that vaginally colonising uropathogens can enter the bladder and cause infection whether they are transiently or persistently colonising the vagina.

SELECTED HOST FACTORS WHICH INFLUENCE VAGINAL MICROECOLOGY

Nonsecretor phenotype

Women with a history of recurrent UTIs are 2 to 4 times more likely to be nonsecretors of histo-blood group antigens than are women without such a history (*Kinane*, 1982; *Sheinfeld* et al., 1989). Children with febrile UTIs caused by *E. coli* also have a significantly higher prevalence of the nonsecretor phenotype than control subjects (*Jantausch* et al., 1994). Further, uro-epithelial cells from women who are nonsecretors show enhanced adherence of uropathogenic *E. coli* compared with cells from secretors (*Lomberg* et al., 1986). It has not been determined whether nonsecretors are at greater risk for pyelonephritis. Recent data suggests that the biochemical explanation for the increased adherence of *E. coli* to nonsecretors' uro-epithelial cells and for their propensity to develop recurrent UTI may be the presence of unique globoseries glycolipid receptors that bind *E. coli* expressing the P and F adhesins. Through extraction of glycosphingolipids from vaginal epithelial cells collected from nonsecretors and secretors, it has been demonstrated that two extended globoseries glycosphingolipids were selectively expressed by epithelial cells of nonsecretors, but not secretors, presumably as a result of sialylation of the gal-globoside precursor glycolipid, which in secretors is fucosylated and processed to ABH antigens (*Stapleton* et al., 1992).

Exposure to spermicides

Nonoxynol-9 is a nonionic surfactant which is the active ingredient in most spermicidal compounds marketed in the United States. It has been found to have *in vitro* antibacterial activity against several sexually transmitted bacteria, viruses and protozoans (*Bolch* and *Warren*, 1973; *Singh* and *Cutler*, 1982; *Asculai* et al., 1978; *Hicks* et al., 1985). *In vitro* studies have also shown that nonoxynol-9 is markedly less active against uropathogenic bacterial and yeast strains (MIC90 ≥25%) than against *Gardnerella vaginalis* strains (MIC90 ≤0.015%) and the *Lactobacillus* strains (MIC90 8%) tested (*McGroarty* et al., 1990; *Hooton* et al., 1991a). Hydrogen peroxide-producing strains of *Lactobacillus* appear to be more susceptible to nonoxynol-9 (MIC90 4%) than non-producers (MIC90 16%) (*Hooton* et al., 1991a). *Escherichia coli* strains which express type 1 fimbriae and vaginal strains of lactobacilli appear to adhere in significantly higher numbers to vaginal epithelial cells pre-incubated with nonoxynol-9 than to control cells (*Hooton* et al., 1991a).

These *in vitro* findings have generally but not always been confirmed in clinical studies. For example, a recent clinical study did not demonstrate nonoxynol-9 to be protective against human immunodeficiency virus, gonorrhoea, or *Chlamydia* (*Roddy* et al., 1998). We evaluated the effects of contraceptive method on the occurrence of bacteriuria and vaginal colonisation with *E. coli* in a study of 104 women who were seen prior to having sexual intercourse, the morning after intercourse, and 24 hours later (*Hooton* et al., 1991b). After intercourse, the prevalence of *E. coli* bacteriuria increased slightly in oral contraceptive users but dramatically in both spermicidal foam and condom users and diaphragm-spermicide users. Twenty-four hours later, the prevalence of bacteriuria remained significantly elevated only in the latter two groups. Similarly, vaginal colonisation with *E. coli* increased, compared with baseline, in all three groups but was more dramatic in the diaphragm-spermicide users (26% at

baseline vs. 61% after diaphragm-spermicide use; p=0.0002) than in foam and condom users (9% vs. 41%; p=0.001) and oral contraceptive users (15% vs. 35%; p=0.03). The effect on vaginal flora was more persistent in users of diaphragm-spermicide and foam and condoms than users of oral contraceptives. Vaginal colonisation with lactobacilli did not change significantly after intercourse in any of the groups.

In another study, sexually-active women were evaluated prospectively over a 6-month period to determine the effects of sexual intercourse and diaphragm-spermicide use on the vaginal microflora (*Hooton* et al., 1994). Two groups of young women, 20 with a history of recurrent UTI (3 or more UTIs in the past year) and 20 without such a history, were selected to determine if sexual and contraceptive practices had different effects in women at different risk for UTI. At visits at which sexual intercourse with diaphragm-spermicide use was reported in the three preceding days, there were marked alterations in vaginal flora as compared with visits preceded by no sex. Thus, the prevalence of *E. coli* colonisation increased from 13% to 59% (p<0.0001); other aerobic Gram-negative uropathogens increased from 4% to 20% (p=0.0045); Group D streptococci increased from 17% to 33% (p=0.014); Group B streptococci increased from 7% to 27% (p=0.0015); and *Candida* species increased from 7% to 40% (p<0.0001). In contrast to the effect of diaphragm-spermicide use on uropathogens and yeast, the prevalence of lactobacilli decreased to 68% in association with recent diaphragm-spermicide use from 87% at visits preceded by no sex (p<0.0001). Fewer visits were preceded by spermicide use without a diaphragm, but the effect on vaginal colonisation with *E. coli* was the same (66% of women were colonised at visits after spermicide use).

Given the differential effects of nonoxynol-9 on lactobacilli and uropathogens described above, it has been suggested that the adverse effects of spermicide on vaginal flora reported in studies such as those described may be in part due to a decrease in lactobacilli, especially protective H_2O_2-positive lactobacilli, after spermicide use. In a recent study of women with (cases) and without (controls) recurrent UTI, it was found that vaginal *E. coli* colonisation was significantly more frequent in cases than controls (35% vs. 11%; p<0.001) and in women without H_2O_2-positive lactobacilli than in women with H_2O_2-positive lactobacilli (odds ratio [OR], 4.0; p=0.01) (*Gupta* et al., 1998). Spermicide use was associated with greater risk of vaginal *E. coli* colonisation (OR, 12.5; p<0.001) and with absence of H_2O_2-positive lactobacilli (OR, 2.9; p=0.04). The inverse association between H_2O_2-positive lactobacilli and vaginal *E. coli* colonisation remained in case patients after controlling for spermicide use (OR, 6.5; p=0.02). Another recent study showed an increase in vaginal coliforms and decrease in vaginal lactobacilli after nonoxynol-9 instillation in the absence of sexual activity or diaphragm use (*Rosenstein* et al., 1998). Women with reduced lactobacilli were less likely to regain normal flora than were those whose lactobacilli were unaffected. However, coliform colonisation occurred whether lactobacilli produced H_2O_2 or not. *Hillier* et al. (1992) also did not find an association between vaginal colonisation with *E. coli* and absence of H_2O_2-producing lactobacilli in their study of pregnant women. Moreover, in a recent study among women using 3.5% nonoxynol-9 gel daily for two weeks, loss of H_2O_2-positive lactobacilli was not observed. However, only 23% of the study women had H_2O_2-positive lactobacilli at baseline suggesting that the study group

had a high rate of abnormal flora at baseline (*Richardson* et al., 1998).

Based on these *in vitro* and clinical studies, it seems likely that the differential antimicrobial activity of spermicides may alter the vaginal ecosystem, provide an environment conducive to the growth of uropathogens and, thus, predispose women who use these products to UTI. Spermicides may provide this selective advantage in colonising the vagina with nonoxynol-9-resistant uropathogens via a reduction in vaginal lactobacilli. Even the relatively small amounts of nonoxynol-9 on coated condoms increase the risk of UTI, presumably by altering flora and facilitating uropathogen colonisation (*Fihn* et al., 1996). Uropathogens from the faecal reservoir that come into contact with the vaginal introitus during insertion of a spermicide, especially if a diaphragm is used concomitantly, may be more likely to persist in the introitus in the presence of nonoxynol-9 because of a reduction in colonisation resistance attributable to a reduction in vaginal lactobacilli (especially H_2O_2-producing strains) and possibly an increased adherence to epithelial cells. Some studies as noted above, however, have not demonstrated that nonoxynol-9 significantly affects H_2O_2-producing lactobacilli. Thus, the mechanism whereby spermicide alters vaginal flora warrants further investigation.

Exposure to antimicrobials

Certain antimicrobials, particularly beta-lactams, can facilitate vaginal colonisation with uropathogens in animals. In a study of adult female cynomolgus monkeys, who carry the Gal-Gal receptor for P-fimbriae, persistent colonisation with P-fimbriated *E. coli* could be obtained in only 4 (17%) of 24 experiments in which the vagina was washed with a suspension of the strain (*Herthelius* et al., 1988). However, a persistent and heavy colonisation of the vagina occurred in 5 of 5 attempts when amoxicillin was administered intravaginally at the same time. Likewise, previous exposure to intravaginal cephadroxil was also shown to promote vaginal colonisation with cephadroxil-susceptible P-fimbriated *E. coli* (*Winberg* et al., 1993). There was a marked decrease in the total number of indigenous vaginal anaerobic bacteria following cephadroxil exposure. Data from these studies and other amoxicillin studies (*Herthelius* et al., 1989a; *Herthelius-Elman* et al., 1992a) suggest that facilitation of *E. coli* colonisation by these antimicrobials may be due to alterations in the indigenous anaerobic flora of the vagina and, thus, altered colonisation resistance. The natural colonisation resistance could not clearly be correlated with the presence of lactobacilli, which were only transiently reduced by amoxicillin. The colonisation resistance against *E. coli* could only partly be restored by vaginal instillation of lactobacilli, but was fully restored by flushing of the whole vaginal flora from a healthy monkey (*Herthelius* et al., 1989b). Trimethoprim and nitrofurantoin, which have much less effect on the periurethral anaerobic flora than does amoxicillin (*Lidefelt* et al., 1990), did not result in enhanced vaginal colonisation with *E. coli* in similar monkey experiments (*Herthelius-Elman* et al., 1992b).

Human data also suggest that certain antimicrobials facilitate susceptibility to UTI. Ampicillin given to adult women with acute cystitis induced a profound reduction in the indigenous genital flora and a concomitant increase in genital *E. coli* colonisation (*Reid* et al., 1990). Amoxicillin given to girls with respiratory tract infections resulted in a dramatic decrease in the peri-urethral anaerobic flora and a concomitant increase in the aerobic gram negative peri-urethral flora which normalised three weeks after therapy (*Lidefelt* et al., 1991). In contrast, ten girls given

trimethoprim-sulfamethoxazole had no major changes in their anaerobic or aerobic Gram-negative microflora during or after therapy. We have found that women with *E. coli* cystitis who are treated with amoxicillin or cefadroxil are more likely to have persistent vaginal and urethral colonisation with *E. coli* and more frequent recurrences of cystitis than women treated with trimethoprim-sulfamethoxazole or fluoroquinolones (*Hooton* et al., 1995). The superior efficacy of trimethoprim-sulfamethoxazole and fluoroquinolones in treating cystitis as determined by a 4 to 6 week follow-up suggests that drugs which eradicate introital *E. coli* while maintaining anaerobic flora are associated with the best outcome.

In a recent prospective study of premenopausal women starting a new contraceptive method, we found that 326 women in a university cohort and 425 women in a health-maintenance organisation cohort were at increased risk for UTI (OR 2.57 and 5.83, respectively) if antimicrobials had been taken during the previous 15 to 28 days but not during the previous 3, 7, or 14 days (*Smith* et al., 1997). The increased risks were noted both for women whose antimicrobial use was for treatment of a previous UTI and for women who received antimicrobials for other illnesses. These results are further convincing evidence that recent antimicrobial use increases a woman's risk of UTI, perhaps by altering the indigenous urogenital flora and predisposing to vaginal colonisation wit uropathogens.

Exposure to oestrogen

In vitro experiments suggest that oestrogens are more likely than progesterones to increase *E. coli* adherence. HeLa cells incubated with increasing concentrations of oestrogens had progressively enhanced attachment of *E. coli*, staphylococci and other bacteria whereas other hormones, including progesterone, had no such effect (*Sugarman* and *Epps*, 1982). *In vitro* studies have also demonstrated that adherence of *E. coli* and other uropathogens to human vaginal or uro-epithelial cells is highest for cells collected during the phase of the menstrual cycle when oestrogen peaks (*Hooton* and *Stamm*, 1996). Animal studies have also shown a peak in adherence of bacteria to vaginal epithelial cells in the pro-oestrus and oestrus of rats, which appeared to be related to oestrogen levels (*Hooton* and *Stamm*, 1996). *Sobel* and *Kaye* (1986) demonstrated significantly increased attachment of *E. coli* to both vaginal and bladder epithelial cells in oestrogenised rats compared with non-oestrogenised rats. Moreover, several studies have shown that oestrogen treatment facilitates experimental UTI in animals (*Hooton* and *Stamm*, 1996). Of note, some studies have not demonstrated variation of adherence to vaginal epithelial cells with uropathogens during the menstrual cycle (*Svanborg-Eden* et al., 1980). Conflicting study results may be due to strain variability in oestrogen-mediated alterations in adherence or to technical differences in the assays.

Human studies have shown that vaginal colonisation with *E. coli* is most likely during and just after the menses (*Hooton* and *Stamm*, 1996). *Sharma* et al. (1987) showed that women administered oral contraceptives had increased adherence of *E. coli* to their uro-epithelial cells compared with adherence to their cells before hormone administration. However, the cyclical variation in adherence, with the peak level just before the midcycle, was maintained after hormone administration, although the difference in the peak and trough adherence level was less after hormone administration. Contraceptives possibly increased the risk of UTI in a general population of post-menopausal women (*Orlander* et al., 1992), although these

results were not controlled for possible increases in sexual activity in pill users. In premenopausal women, oral contraceptive use appears not to be associated with an increased risk of UTI (*Strom* et al., 1987).

To evaluate a possible association between UTI and the menstrual cycle in women, we studied 577 women enrolled in antimicrobial treatment trials for acute cystitis (*Hooton* et al., 1996b). Patients were administered a standardised questionnaire which asked for the date of onset of the last menstrual cycle (LMP). Women were significantly more likely to present with UTI 8 to 15 days after the onset of their LMP, which is generally the time of peak oestrogen secretion, than at any other time of the cycle (41% presented during this interval) ($p<0.001$). This association was true for women with UTI caused by *E. coli* (41% presented 8 to 15 days after onset of their cycle; $p<0.001$) and for those with UTI caused by *S. saprophyticus* (47% presented 8 to 15 days after onset of their cycle; $p<0.001$). These data demonstrate a strong association between the time at which women present with acute cystitis and the time from the onset of their last menstrual period. We were not able to determine whether this association was due to a hormonal mechanism or to changes in sexual behaviour in relation to the menstrual cycle (James, 1971; *Spitz* et al., 1975; *Hedricks* et al., 1987).

Recent studies in postmenopausal women further support an association between hormonal status, vaginal flora and UTI. *Raz* and *Stamm* (1993) studied 93 postmenopausal women with a history of recurrent UTI in a randomised, double-blind, placebo-controlled trial of a topically applied intravaginal oestriol cream and evaluated patients serially monthly for 8 months. The incidence of UTI in the group given oestriol was significantly reduced compared with that in the placebo group (0.5 vs. 5.9 episodes per patient-year, $p<0.001$). Lactobacilli were absent in all vaginal cultures before treatment and reappeared after one month in 61% of the 36 oestriol-treated women compared with none of 24 placebo recipients ($p<0.001$). The prevalence of *Enterobacteriaceae* fell from 67% to 31% in oestriol recipients but was virtually unchanged in the placebo recipients ($p<0.005$). There appeared to be a relation between vaginal colonisation with lactobacillus and UTI in that 3 of 23 oestriol-treated women who were colonised with lactobacillus after therapy developed UTI compared with 7 of 13 who were not colonised. Although these findings appear to contradict the findings noted above in premenopausal women, any adverse effects of oestrogen in postmenopausal women may be overshadowed by oestrogen's effect on restoration of indigenous lactobacilli to the vaginal environment and reduction of colonisation and infection with uropathogens, perhaps by lowering pH, production of bactericidal substances, or competitive exclusion of uropathogens from uro-epithelial cells.

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THE VAGINAL FLORA IN IDIOPATHIC REPRODUCTIVE TRACT DISEASES OF WOMEN AND AN ANIMAL MODEL

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SUMMARY

This paper describes the results of a survey of over 380 women of child-bearing age who were assessed for the cause of their respective pelvic inflammatory complaints or women who presented without health complaints. The data show a strong correlation between infection with *Ureaplasma urealyticum* and chronic vaginal discharge and inflammation in the presence of an apparently normal population of vaginal lactobacilli. A suggestion of association with complaints consistent with endometriosis with *U. urealyticum* infection is noted and a possible murine model for this disease is presented.

INTRODUCTION

I wish to present some work done over a period of years, first with Miles Laboratories and then at the Trudeau Institute in Saranac Lake, New York, and also mention something dear to the hearts of Prof. David Taylor-Robinson and me: An animal model for urogenital mycoplasmosis.

The original objective of the program and team I led was to develop an antibody-based diagnostic kit that could be used in a clinician's office to diagnose gonorrhoea and other common causes of pelvic inflammatory disease (PID). A subsequent objective was to ascertain whether ureaplasmas had a causal relationship to endometriosis, a subject I would like to hear discussed during the symposium although there are no hard data in the literature yet to support such a relationship.

The approach we used was to collect vaginal fluids from patients with suspected gonorrhoea, undiagnosed vaginal infections, or women free of vaginal infection, according to the clinical judgement of the physician. The objective was to develop an antibody-based diagnostic test which is why the focus was originally on the vaginal washout fluids.

METHODS

The clinicians were provided with a kit of four 6" sterile cotton swabs, two glass slides and a slide mailer, a disposable 10 cc syringe and a tube containing azide to prevent destruction of protein (antibody), sterile pyrogen-free saline, and bacteriological media (Transgrow for gonococci, Kupferberg's for trichomonads, Nickerson's for *Candida*, and Shepard's for ureaplasmas/mycoplasmas). Assessments were also made, based on patient histories, which

Table 1: Sample population (all patients were aged 15-50 years)

161 patient samples from 3 Ob/Gyn clinics in northern Indiana
170 patient samples from 1 Ob/Gyn clinic in northern New York
58 patient samples from a County Health Department clinic in Indiana

are not the subject of this paper and will not be presented. Maurice Shepard advised on the cultivation of the mycoplasmas (some in the audience may remember that Dr. Shepard's original work on ureaplasmas was done on U.S. Marines at Camp Lejeune in North Carolina).

The procedure was to ask the clinicians to take the specimens, including cervical mucus specimens, put each of two swabs directly into Shepard's or Kupferberg's media, use a third swab to directly inoculate the Transgrow and Nickerson's media and maintain the media at room temperature until processing later in the day. One swab was used to streak each of two slides that were to be Gram-stained or used in fluorescent antibody assays. For the vaginal wash fluid, the physicians were asked to gently irrigate the vagina with 10 cc of saline and collect the fluid into the tube containing azide; this fluid was then refrigerated until processing. The immunological aspects of the study were not as successful as had been hoped and these data are not included in this presentation. The sample population included 161 women seen at three OB/GYN clinics in northern Indiana and 58 patients seen at a county health department clinic, where we saw most of the gonorrhoea. In subsequent studies in association with an OB/GYN clinic in northern New York, we had 170 patients sampled. The patient population ranged in age from 15 to 50; most of the women were in their twenties and thirties.

RESULTS

The sample population is summarised in Table 1 and the number of gonorrhoea cases are shown in Table 2; these are not extraordinary. Sixty cases of candidosis were observed and sixteen cases of trichomoniasis, two of which had a mixed *Candida* and *Trichomonas vaginalis* infection (Table 3). Sixty-six patients had no complaints; these were individuals who came into the clinic for PAP smears, annual physicals, or were in the early stages of pregnancy. Thirty-seven of the patients in the population presented with vaginitis/cervicitis as clinically diagnosed.

With respect to the subject of our symposium, it was interesting that *Candida*, unlike *Trichomonas*, gonorrhoea, and other PID agents which were present with a mixed vaginal flora, 60% of the *Candida* cases were observed in women in whom there was a rich popu-

Table 2: Gonorrhoea cases

11 (58) GC cases from Indiana Health Dept.
5 (161) GC cases from Indiana clinics
8 (170) GC cases from NY clinic

Table 3: Pelvic inflammatory disease (Indiana clinics)

- 60 cases of candidosis and 16 cases of trichomoniasis (2 patients with double infections)
- GC virtually limited to county health clinic
- 66 patients presented with no complaint
- 137 patients presented vaginitis/cervicitis

lation of lactobacilli. What was most interesting to me was the observation that, in the presence of a normal population of lactobacilli, and no other known PID agents, women with a chronic vaginal discharge were positive for ureaplasmas (39%) confirmed by culture (Table 4). This observation was so consistent that we were able to predict, on the basis of observing rich populations of lactobacilli (not a mixed culture of Gram-positive and Gram-negative microorganisms) and unusual numbers of granulocytes on Gram-stains, which patients would show *Ureaplasma* on culture. There was, in fact, a 100% correlation with this observation. The *Ureaplasma*, which prefer a low pH, was in its best environment when there was a good population of *Lactobacillus* in the vagina. Data were also collected on age of the patient, whether the individual used birth control and what type, the number of pregnancies and live births, etc. These data are not presented because there was no correlation with these parameters and the main point of this presentation: That is, that all patients presenting with vaginal discharge in the presence of normal levels of lactobacilli and no *Candida*, were positive for ureaplasmas. Other patients with vaginal discharge for the most part presented with a mixed flora, most often containing members of the Enterobacteriaceae, a finding which so many others have observed. In the study in New York, looking at the incidence of ureaplasmas, a high proportion of infected patients complained of symptoms consistent with endometriosis. We wondered, therefore, whether there could be a causal relationship; such a relationship between *Ureaplasma* infection and endometriosis had not been proven by us or anyone else to date. One of the problems is that endometriosis is very much a clinical diagnosis with confirmation only by the post-mortem or post-hysterectomy pathological observation of the endometrium invading into the myometrium. The palpation of chocolate bodies in the cul de sac is considered diagnostic but occurs only in a small percent of patients. The problem is defining endometriosis in patients which do not present with such clinical findings which has made study difficult. A broad definition of endometriosis usually includes mostly young women, never before pregnant, who complain of chronic dysmenorrhoea. One woman presenting with years of dysmenorrhoea and a chronic infection with *Ureaplasma*, in the absence of other recognised vaginal

Table 4: PID and Flora

- *Candida* observed with lactobacilli in 60% of cases.
- GC and *Trichomonas* observed with mixed flora.
- Normal *Lactobacillus* populations, but vaginal discharge, and none of above, were positive for *Ureaplasma* (39% of remainder).

Figure 1: The reproductive tract from a breeding LEWIS female rat showing cystic ovaries as a result of bilateral salpingitis caused by *Mycoplasma pulmonis* infection.

pathogens, and was placed on hormonal therapy, was followed through her first pregnancy. A deep tissue specimen of the placenta, not contaminated by the vaginal flora, was obtained immediately following delivery and *Ureaplasma urealyticum* isolated. The baby was of normal weight, etc. and the gestation period was normal, or slightly extended. This observation led us to consider the role of the mycoplasmas/ureaplasmas in endometriosis and perhaps infertility which may have an animal equivalent, as described below.

MURINE MYCOPLASMOSIS

In a large colony of SPF Lewis rats (LEW), a disruption of the normal breeding colony productivity was seen over many months. This breeding problem was noted in the presence of chronic respiratory disease which was confirmed to be caused by *Mycoplasma pulmonis* (*Cassel* et al., 1976). Initially, we viewed the breeding problems as due to poor health in the rat colony as a result of the respiratory disease and attempts were made to treat this disease. However, necropsies of older breeding females disclosed the presence of ovarian cysts (Figure 1), the fluid from which contained pure cultures of *M. pulmonis*, and unilateral or bilateral salpingitis. The cysts and the associated salpingitis was clearly the cause of the drop in fertility, or complete infertility in

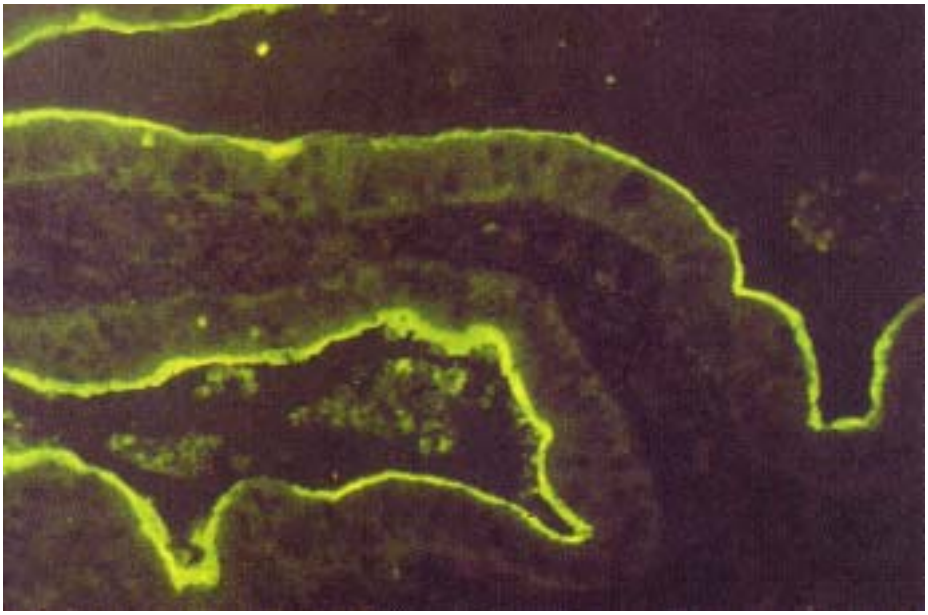

Figure 2: Colonisation of the uterine epithelium of Lewis rats by pure *M. pulmonis* (determined by fluorescence antibody).

the case of bilateral salpingitis. Histological examination showed colonisation of the uterine epithelium which proved to be pure *M. pulmonis*, determined by fluorescence antibody (Figure 2). The chronic infection of the reproductive tract led to metaplasia of the epithelium and even erosion. Curing the colony of *M. pulmonis* through caesarean section into a gnotobiotic environment resulted in a complete resolution of the breeding problem. While this is an animal model that is not the best reflection of what we currently know of the human situation, it does provide a pathogenic mechanism for the possible aetiology of endometriosis due to mycoplasmas or ureaplasmas. These organisms form very close associations with epithelial cell membranes, even penetrating them, and results in metaplastic changes which may permit such cells in the endometrium to invade into the myometrium causing the clinical presentation we call endometriosis.

DISCUSSION

There is controversy over whether ureaplasmas are frank pathogens able to cause clinical disease in women. Although it is quite well accepted that *Ureaplasma urealyticum* can cause nongonococcal urethritis (NGU) in men as a pure culture inoculated into volunteers, even its role in normally acquired NGU is being questioned. Data presented here argue for a role in PID since the organisms were consistently found in the presence of low vaginal pH, a rich population of lactobacilli and no other bacterial pathogens. Although it was not possible to exclude infection by *Chlamydia*, there are no reports of chlamydial infections of such high incidence which would totally account for

our observations. Methods to assess *Mycoplasma genitalium* infection were not available at the time of this work and infection with this organism cannot be excluded. Recent studies by others with this organism would suggest a direct role in PID and would be similar to the disease reported here in rats associated with a species of Mycoplasma. But again, the known incidence of *M. genitalium* in western populations would argue against it being the sole cause of the PID in our sample group.

CONCLUSIONS

This paper describes the results of a survey of over 380 women of childbearing age who were assessed for the cause of their respective pelvic inflammatory complaints or women who presented without health complaints. The data show a strong correlation between infection with *Ureaplasma urealyticum* and chronic vaginal discharge in the presence of an apparently normal population of vaginal lactobacilli. A suggestion of association with complaints consistent with endometriosis with *U. urealyticum* infection is noted. A rat model for the study of reproductive tract disease in humans caused by *Mycoplasma* is presented. The pathology observed in the LEW rat bears many similarities to human PID and may provide a controlled system for the assessment of pathogen virulence factors, pathogenesis and immunogenesis in PID.

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UREAPLASMA UREALYTICUM AND *MYCOPLASMA HOMINIS* IN PREGNANCY AND OBSTETRIC OUTCOME

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SUMMARY

The roles of the genital mycoplasmas *Ureaplasma urealyticum* and *Mycoplasma hominis* in infection-mediated preterm birth, and preterm prelabour rupture of the membranes (PPROM) are reviewed. Studies of vaginal flora during pregnancy and in labour provide conflicting data concerning the possible association between the presence of these microorganisms in the vagina and poor obstetric outcome. However studies of the chorioamnion have clearly established the link between these microorganisms, preterm labour and PPROM. These studies of the chorioamnion show *U. urealyticum* is the most common microorganism found in the chorioamnion and is associated with intrauterine infection, chorioamnionitis, very low birthweight and membrane rupture.

U. urealyticum occurs in increased concentrations in women with bacterial vaginosis but in a prospective study of vaginal flora at 24 weeks gestation there was an additive attributable risk for preterm birth for *U. urealyticum* greater than that due to bacterial vaginosis. However preventative strategies should focus on prevention of bacterial vaginosis.

M. hominis has a lower prevalence in normal vaginal flora (5-10%) than *U. urealyticum* (24-82%), but also occurs in increased concentrations in bacterial vaginosis. Vaginal *M. hominis* in pregnancy is associated with preterm birth, generally in the presence of bacterial vaginosis. It is also a cause of post-partum and post-abortal infection.

Host factors play an important role in determining pregnancy outcome in women with *U. urealyticum* and *M. hominis* ascending infection. Host response studies are required to identify subgroups of women who are at a higher risk of adverse pregnancy outcome, thus enabling strategies for prevention of infection-mediated preterm labour to be formulated. Screening and treatment for *U. urealyticum* and *M. hominis* in pregnancy is not recommended, as their role in preterm birth is linked with the bacterial vaginosis micro-ecology.

INTRODUCTION

Ureaplasma urealyticum and *Mycoplasma hominis* have been associated with infections in pregnancy and labour for more than twenty years, yet their role in infection-mediated preterm birth is still somewhat controversial. Recent

studies have provided further understanding of their pathological role in adverse pregnancy outcome. Preterm labour and delivery has remained constant at 7-10% in the western world, despite significant advances in obstetric medicine, and the aetiology is still not fully understood. With improved neonatal care 85% of very low birthweight babies now survive, however a number have considerable residual morbidity. Evidence is continuing to accumulate that subclinical intrauterine infection is present in a significant proportion of women in preterm labour. Ascending vaginal microorganisms, including the genital mycoplasmas, may cause inflammation and weakening of the membranes, and subsequent invasion of the amniotic fluid. The presence of subclinical infection is associated with increased production of various cytokines in the amniotic fluid, preceding labour. Better understanding of the pathogenesis and aetiology of the genital mycoplasmas in pregnancy and labour may enable strategies for prevention of mycoplasma-associated preterm birth and preterm prelabour rupture of membranes (PPROM) to be implemented.

This is not an exhaustive review of the literature but a brief review to consider evidence for the role of *U. urealyticum* and *M. hominis* in intrauterine infection and preterm birth, and vaginal colonisation as a risk factor.

REVIEW AND DISCUSSION

Mycoplasmas are the smallest free-living, self-replicating organisms known. Twelve species occur in humans, three in the genital tract, *U. urealyticum*, *M. hominis* and *M. genitalium*. *U. urealyticum* and *M. hominis*, known as genital mycoplasmas, colonise the vagina as part of the normal vaginal flora. *M. hominis* and *U. urealyticum* occur in the vagina of 5 to 10 percent and 24 to 82 percent of women of reproductive age respectively (*Carey* et al., 1991; *Cassell* et al., 1993; *McDonald* et al., 1992). There is a close relationship between hormonal status and the occurrence of genital mycoplasmas. *U. urealyticum* prevalence increases with younger age, lower socio-economic status, multiple sexual partners, black ethnicity, and oral contraceptive use (*Eschenbach* 1993). One of the first reports of amniotic fluid infection with genital mycoplasmas was published in 1983 by *Cassell* and co-authors who reported amniotic fluid infection at 16 to 20 weeks' gestation with *M. hominis* and *U. urealyticum* without rupture of the foetal membranes. Since then there have been a number of reports of clinically silent, chronic intrauterine infection with *U. urealyticum* and intact membranes, often during the mid-trimester. The nature and timing of this chronic intrauterine infection appears to be different to that caused by virulent organisms such as group B Streptococcus and *Escherichia coli*, which provoke an intense and clinically apparent chorioamnionitis and rapid onset of labour.

Placental Studies

The recovery of microorganisms from the chorioamnion (i.e. swabs taken aseptically from between the chorionic and the amnionic membranes) is associated with chorioamnionitis (*Hillier* et al., 1988). Microorganisms obtained from this site represent a true infection, not just vaginal contamination (*Eschenbach*, 1993). *U. urealyticum* was isolated from the chorioamnion of 38-66% of women with histological chorioamnionitis and from 13-17% without histological chorioamnionitis (*Hillier* et al., 1988; *Kundsin* et al.,

1984; *Embree* et al., 1980). A variety of microorganisms may be recovered from the chorioamnion including *U. urealyticum* and *M. hominis* but *U. urealyticum* is the most frequent isolate. The recovery of *U. urealyticum* from amniotic fluid was also associated with chorioamnionitis in four of five recent studies (*Gray* et al., 1992; *Horowitz* et al., 1995; *Montuclard* et al., 1996; *Kerki-Nisula* et al., 1997; *Yoon* et al., 1998).

A retrospective four year review of 122 autopsy and placental cultures from spontaneous, unexplained miscarriages and stillbirths between 16 to 26 weeks found that *U. urealyticum* and group B Streptococcus were the most common isolates (*McDonald* and *Chambers*, unpublished results). *U. urealyticum* was more common in the placenta than foetal tissue. In cases with histological chorioamnionitis, *U. urealyticum* was found in 24 percent compared with 8 percent in cases with no such evidence. *U. urealyticum* was also more common in women with ruptured membranes, however it is impossible to know whether this was post rupture invasion of the chorioamnion or whether *U. urealyticum* played a part in weakening and eventual rupture of the membrane. The association of *U. urealyticum* with PPROM in women in the last month of pregnancy in a prospective study of 577 pregnancies (*Jacqui* and *Sedallian*, 1992) and the prospective study of *McDonald* et al. (1992) detailed below, provides further support for this view.

Studies on the relationship between the isolation of genital mycoplasmas from the chorioamnion and low birthweight show that the lower the birthweight the higher the recovery of *U. urealyticum* from the placenta (*Embree* et al., 1980; *Kundsin* et al., 1984). Of six studies looking at isolation of *U. urealyticum* from the placenta and preterm birth, three supported an association between *U. urealyticum* and preterm birth (*Hillier* et al., 1988; *Kundsin* et al., 1984; *Embree* et al., 1980), and three did not (*Naessens* et al., 1989; *Zlatnik* et al., 1990; *Hillier* et al., 1991). The answer to this apparent discrepancy may lie in differing population subgroups in the various studies. In a recent study *U. urealyticum* from the chorioamnion was associated with preterm birth before the 29th week of gestation and with increasing duration of time between rupture of membranes and delivery (*Kundsin* et al., 1996).

Studies of Vaginal Flora During Labour

In several studies vaginal colonisation with *U. urealyticum* and *M. hominis* at time of labour has been associated with preterm birth. Ureaplasmas were isolated from 86% of women who gave birth at less than 34 weeks gestation compared with 46% of a similar gestation who were not in preterm labour, and heavy colonisation with *M. hominis* was detected in 18% compared with 0%, respectively (*Lamont* et al., 1987) (Tables 1 and 2). A recent study by *Abele-Horn* et al. (1997) also showed a significantly higher rate of preterm birth, PPROM and chorioamnionitis in women with *U. urealyticum* in labour. The finding that an association existed between heavy colonisation with *M. hominis* but not the presence of *M. hominis*, and preterm birth (*Lamont* et al., 1987) supports the hypothesis that it is the concentration of *M. hominis* in the vagina that is important rather than just the presence of this organism. However other studies showed no associations between either *U. urealyticum* (*Martius* et al., 1988; *McDonald* et al., 1991) or *M. hominis* (*McDonald* et al., 1991) in labour.

Table 1: Evidence linking vaginal *M. hominis* in pregnancy to obstetric outcome

Reference	Year	Gestation	n	Outcome OR/RR (95%)
Braun et al.	1971	First visit	485	n.s.
Ross et al.	1981	Three visits	162	n.s.
Upadhyaya et al.	1983	First visit	135	n.s.
Harrison et al.	1986	First visit	3,224	n.s.
Lamont et al.	1987	Labour- Heavy colonisation		PTB<34 weeks, p<0.05
Sweet et al.	1987	First visit	3,293	n.s.
Polk et al.	1989	22-30 weeks	801	PTB, RR 2 (90% CI 1.42-2.93)
McGregor et al.	1990	24 weeks	202	PTB, RR 5.1 (1.45-17.9)
Carey et al.	1991	22-26 weeks	4,934	n.s.
McDonald et al.	1992	24 weeks	786	n.s.
Jacqui, Sedallian.	1992	Last month	577	PPE, p<0.05
Divers & Lilford	1993	Meta-analysis		PTB, p<0.05
Germain et al.	1995	23-26 weeks	13,914	IUGR, RR 1.16 (1.04-1.29)
Hillier et al.	1995	23-26 weeks	10,397	BV + *M. hominis* RR 1.6 (1.1-2.3)

Studies of Vaginal Flora During Pregnancy

Given the indications that in at least some women ascending genital tract infection with *U. urealyticum* or *M. hominis* may be a cause of preterm labour and PPROM, the question which must be answered is: "Are women with vaginal colonisation with these organisms during pregnancy at higher risk of adverse pregnancy outcome?" In order to answer this question several studies of vaginal flora in early and mid-pregnancy have investigated possible associations between the microorganisms found in vaginal flora and adverse pregnancy outcome. The mycoplasma findings for some of these studies are listed in Tables 1, and 2.

Following our study of vaginal flora in preterm and term labour, we undertook a prospective study of vaginal flora at 24 weeks gestation to investigate whether the carriage of any particular organism during mid trimester placed a woman at higher risk of preterm birth (*McDonald* et al., 1992). As the causes of preterm birth are multi-factorial it is essential that multiple logistic regression analysis be performed to take account of demographic and obstetric variables known to place a woman at increased risk of preterm birth. Also several studies have only focused on one or a few microorganisms and not taken into account the possible association of other organisms. 786 women were cultured for aerobes, anaerobes and mycoplasmas using three high vaginal swabs at approximately 24 weeks gestation. *U. urealyticum* and heavy growth of *Gardnerella vaginalis* were the only two microorganisms found to be associated with preterm birth. Of importance in this study was the finding that *U. urealyticum* was also associated with a three-fold increased risk of PPROM. This confirmed the study of *Minkoff* et al. (1984) who found that *U. urealyticum* but not *M. hominis* was associated with preterm labour. However *Polk* et al. (1989) and *McGregor* et al (1990) found *M. hominis,* but not *U. urealyticum,* was associated with in-

Table 2: Evidence linking vaginal *U. urealyticum* in pregnancy to obstetric outcome

Reference	Year	Gestation	n	Outcome OR/RR (95%)
Braun et al.	1971	First visit	485	n.s.
Ross et al.	1981	Three visits	162	n.s.
Upadhyaya et al.	1983	First visit	135	n.s.
Minkoff et al.	1984	First visit	220	PTL, RR 1.33 ($p<0.05$)
Harrison et al.	1986	First visit	1587	n.s.
Lamont et al.	1987	Labour		PTB<34 weeks, $p<0.05$
McGregor et al.	1990	24 weeks	202	n.s.
Carey et al.	1991	22-26 weeks	4,934	n.s.
McDonald et al.	1992	24 weeks	786	PTB, OR 1.7 (1.1-2.6) PPROM, OR 3.2 (1.7-6.1)
Jacqui, Sedallian.	1992	Last month	577	PPROM, $p<0.05$
Joste et al.	1994	First trimester abortion	63	Early abortion, $p<0.05$
Chua et al.	1994	13-34 weeks	312	n.s.
Germain et al.	1995	23-26 weeks	13,914	IUGR, RR 1.2 (1.05-1.38)
Abele-Horn	1997	Labour	253	PTB, $p<0.001$ PPROM, $p<0.001$

creased risk of preterm birth. Four other prospective studies in pregnancy showed no significant associations with adverse pregnancy outcome (*Braun* et al., 1971; *Ross* et al., 1981; *Upadhyaya* et al., 1983; *Harrison* et al., 1986). The incidence of *M. hominis* and *U. urealyticum* varied from 5 to 47 % and 44 to 79% respectively in these studies which may account for the disparity in results.

In the Vaginosis in Pregnancy Trial 10,397 women were studied at 23 to 26 weeks gestation (*Hillier* et al., 1995). Among women with bacterial vaginosis the highest risk of preterm birth was found in those with both bacterial vaginosis and *M. hominis.* In addition 4,934 women were evaluated for carriage of *U. urealyticum.* After multivariate analysis no association was found with preterm birth or PPROM (*Carey* et al., 1991). However women with other pathogens as well as *U. urealyticum,* such as group B streptococcus and *Chlamydia trachomatis*, were excluded from analysis.

In the study of *McDonald* et al. (1992), the separate risk for preterm birth attributable to *G. vaginalis* was 9% while 24% may be explained by colonisation with *U. urealyticum.* The joint risk attributable to *G. vaginalis* and *U. urealyticum*, after allowing for the effects of previous preterm delivery and multiple pregnancy, was 26%. The *G. vaginalis* and *U. urealyticum* attributable risks were not independent, as 7% of women were colonised with both organisms but there was an independent attributable risk for carriage of *U. urealyticum* over and above the joint attributable risk. This indicates that there is an additional component of risk for preterm birth over and above the risk from bacterial vaginosis.

A meta-analysis (*Divers* and *Lilford*, 1993) of studies available at that time found an overall significantly increased risk of preterm birth with *M. hominis* colonisation during pregnancy. In addition, since 1993 the results of the VIP study showed an increased risk of preterm birth if *M. hominis* was present

in women with bacterial vaginosis. In summary there is considerable evidence indicating a role for high concentrations of *M. hominis* in the vagina in early to mid pregnancy, in infection-mediated preterm birth. This appears to be in conjunction with bacterial vaginosis.

The role of *U. urealyticum* in pregnancy is less well defined. Despite the negative findings of the VIP study, five other studies since 1989 have shown associations with adverse pregnancy outcome (*McDonald* et al., 1992; *Jacqui* and *Sedallian*, 1992; *Joste* et al., 1994; *Germain* et al., 1995; *Abele-Horn* et al., 1997), and this is also reflected in the findings of the placental studies. It may be there are one or more subgroups of pregnant women at risk for ascending infection with the genital mycoplasmas. In severe infections a serological response to *U. urealyticum* can sometimes be measured and it may be that women who lack antibodies to these organisms are at higher risk of ascending infection. Alternatively, certain clinico-pathological conditions may place a woman at risk. If only a proportion of women are at risk of infection, this may explain why a number of prospective studies of vaginal flora in pregnancy do not show an association between *U. urealyticum* in pregnancy and adverse pregnancy outcome.

PATHOGENESIS

U. urealyticum:

U. urealyticum is unique among the mycoplasmas in its ability to metabolise urea through the enzyme urease. Cells of *U. urealyticum* attach to erythrocytes and other eucaryotic cells, and produce a haemolysin which lyses erythrocytes. Like other bacteria associated with preterm birth, *U. urealyticum* is known to produce phospholipase A_2, an enzyme which frees bound arachidonic acid from foetal membrane cells, resulting in a cascade of prostaglandin synthesis that leads to uterine contractions. IgA protease activity has been demonstrated for *U. urealyticum*, and this is thought to be an important virulence factor of mucosal pathogens. The influence of hormones upon vaginal colonisation was demonstrated in studies in female mice in which treatment with oestradiol enabled colonisation to occur (*Furr* and *Taylor-Robinson*, 1989). It has been shown that *U. urealyticum* by itself can produce chorioamnionitis, although many chorioamniotic infections occur mixed with other organisms, especially bacterial vaginosis organisms, indicating a symbiosis. *U. urealyticum* is thought to be a cause of chronic lung disease in the very low birthweight neonate (*Cassell* et al., 1993). It has also been associated with non-gonococcal urethritis, pelvic inflammatory disease, infertility, septic arthritis and urinary stone formation. Two biovars, parvo and T960 have been determined in pregnant women. Although biovar parvo was more common, T960 was dominant in women with preterm birth and miscarriage (*Abele-Horn*, 1997). Fourteen serotypes of *U. urealyticum* are known but only some are involved in disease.

M. hominis:

M. hominis organisms can adhere to many eucaryotic cells. Like *U. urealyticum*, colonisation of the genital tract of mice by *M. hominis* is dependent on oestradiol, but not progesterone, hormone treatment, (*Furr* and *Taylor-Robinson*, 1989). High concentrations of *M. hominis* occur in some women with bacterial vaginosis. Heavy *M. hominis* colonisation, generally in the presence of bacterial vaginosis, is associated with preterm birth. In addition to

genital tract infections, post partum and post abortal fever, *M. hominis* has been found in a number of blood, joint, wound, central nervous system and respiratory infections, generally in debilitated, immunosuppressed or neonatal patients. *M. hominis* is a potent stimulator of neutrophil chemoattractant cytokines in alveolar type II cells.

PREVENTION STRATEGIES AND *U. UREALYTICUM* ERYTHROMYCIN TREATMENT TRIALS

If there is an association between vaginal carriage of the genital mycoplasmas during pregnancy and adverse pregnancy outcome, then formulation of strategies for prevention of preterm birth may be possible. However, eradication of *U. urealyticum* vaginal colonisation is very difficult to achieve. Treatment with oral erythromycin at 29 weeks' gestation was not successful in eliminating *U. urealyticum* from the vagina in a randomised, double-blind trial to prevent preterm delivery (*Eschenbach* et al., 1991). After four weeks of erythromycin or placebo, recovery of *U. urealyticum* from the vagina was no different (79% vs. 84%). This is not surprising as erythromycin would not be expected to be effective at the low pH of the vaginal mucosa. Also it is very difficult to eradicate any organism present as mucosal normal flora, as these organisms are usually not in an actively replicating stage. Finally the gestation of treatment in this study was too late to prevent ascending infection. If treatment is to have any chance of preventing ascending infection it should be instituted early in pregnancy before organisms colonise the endometrium.

TREATMENT IN PRETERM LABOUR

A number of studies have treated women in preterm labour with erythromycin in an attempt to prevent delivery. Whilst some have shown an increase in time to delivery of several days when compared with controls, we do not know how effective erythromycin is in eradicating *U. urealyticum* from the placenta, and we know that erythromycin does not penetrate the amniotic sac (*Cassell* et al., 1993). Therefore other preventative strategies must be sought to prevent ascending infection in susceptible women.

HOST FACTORS AND IDENTIFICATION OF SUBGROUPS AT RISK

Why do some women develop ascending infection and others do not? The current urgent requirement is the identification of which particular women are at risk of ascending infection. If a specific subgroup of women can be identified, strategies can then be focused upon this group of women rather than all pregnant women, with a correspondingly much greater chance of success. A recent study analysed a range of demographic and other variables but failed to identify any particular population subgroup at higher risk of adverse pregnancy outcome when *U. urealyticum* colonisation was present (*Eschenbach* et

al., 1991). Other studies have indicated lack of specific protective antibody may be a risk factor, e.g. for group B Streptococcus.

Current studies on cytokine production during pregnancy seek to identify women at risk and women in whom an infectious process has already begun. Other studies on the immune response aim to identify women at risk before infection has commenced, i.e., those who are more susceptible to infection. However the aetiology of preterm birth is multi-factorial and we would expect a number of host factors may be involved.

CONCLUSIONS

In conclusion we know that intrauterine infection with *U. urealyticum* and *M. hominis* can occur via ascending genital tract infection and this is associated with chorioamnionitis. We also know that infection of the chorioamnion with these microorganisms is associated with preterm birth, spontaneous abortion, miscarriage and PPROM. There is evidence that *M. hominis* vaginal colonisation, generally in the presence of bacterial vaginosis, increases the risk of infection-mediated preterm birth. There is an additional attributable risk for preterm birth for vaginal *U. urealyticum* in addition to the joint attributable risk for bacterial vaginosis, although there does not appear to be a significant association between vaginal *U. urealyticum* and preterm birth.

Host factors are equally as important as microbial factors in determining pregnancy outcome, yet these have not been as well studied to date. Further studies need to be undertaken, especially studies of the local immune response and host response factors.

Finally screening and treatment for *U. urealyticum* and *M. hominis* in pregnancy is not recommended, as their role in preterm birth is linked with the bacterial vaginosis micro-ecology. If screening for vaginal infections is being considered in women at risk of preterm birth, then screening and treatment for bacterial vaginosis may be undertaken, as eradication of bacterial vaginosis should also reduce the risk of *U. urealyticum* and *M. hominis* ascending infection.

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TREATMENT OF VAGINAL INFECTIONS WITH LACTOBACILLI: A CLINICIANS REVIEW

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SUMMARY

Bacterial vaginosis (BV) and vaginal candidosis are the most common diagnoses in women with symptoms from the lower genital tract. Treatments with antibiotics and antifungals are effective but are impaired by side effects. A considerable number of women suffer recurrences and have a need for alternative treatments.

Treatment of vaginal ailments with lactobacilli has a long tradition. Published reports indicate that BV and candidosis may be treated with lactobacilli but additional studies on the microbiology of lactobacilli as well as further clinical investigations are needed to elucidate all aspects of such treatments.

INTRODUCTION

Symptoms from the lower genital tract like dysuria, itching and discharge are common in women attending outpatient clinics. Most often they are caused by a vulvo-vaginal infection (*Komaroff* et al., 1978). There are three common causes for vulvo-vaginal infections in women of childbearing age: candidosis, bacterial vaginosis (BV) and trichomoniasis. The relative frequencies of these infections differ considerably between and also within countries depending on the clinical situation. Since treatment of vaginal infections with lactobacilli has been reported only for candidosis and bacterial vaginosis this review will deal only with these infections.

The diagnosis and treatment of vaginitis has recently been reviewed (*Sobel*, 1997). Standard treatment of vaginitis is with antimicrobials or antifungals. In most clinical situations they perform perfectly. However they are not without complications. Moreover some women suffer from frequent recurrences (*Sobel*, 1997). In some clinical settings there seems to be an increasing number of women with recurrent vaginal candidosis (*Hallén*, 1998). Some of these women encounter problems with repeated treatments. There is also an emerging problem with resistance to azole antifungals in *Candida* strains (*Hallén* et al., 1998). Particularly in these women there is a need for alternative treatments. With the increasing public awareness of the drawbacks of antibiotic treatments many women demand alternative cures.

Treatment of vaginal infections with lactobacilli has a long tradition starting with Döderlein's descriptions of the vaginal flora (*Döderlein*, 1892). The interest in this option has increased during the last ten years (*Redondo-Lopez* et al., 1990; *Reid* et al., 1990; *McGroarty*, 1993; *Elmer* et al., 1996).

Over the years there are a considerable number of reports published on treatment of vaginal infections with different forms of lactobacilli (*Mohler* and *Brown*, 1933; *Butler* and *Beakley*, 1960; *Spitzbart*, 1968; *Gunston* and *Fairbrother*, 1975; *Collins* and *Hardt*, 1980; *Gerstner* and *Müller*, 1987). With today's conception of vaginal infections these studies are of very limited value for the clinician. Information on diagnostic criteria used is lacking or diagnoses are very ill defined and the different kinds of lactobacilli used are most often not characterised in any way.

TREATMENT OF CANDIDOSIS

There are two clinical studies published on the treatment and prophylaxis of vaginal candidosis. Both used commercial yoghurt orally ingested as active treatment.

Hilton and co-authors (1992) designed a crossover study for one year including 33 patients with recurrent vaginal candidosis (≥5 episodes per year; eight are called chronic infections). They were supposed to start either in the active arm with eight ounces of yoghurt daily for six months and then switch to a yoghurt free diet during the remaining six months or vice versa. However the protocol had to be amended due to dropouts (12 protocol violations and two with other infections) but mainly because women in the active arm refused to switch over to yoghurt free diet. Finally only 13 women, aged 24 to 50 years, completed the study and 11 of these started on the control arm. There were no diabetics among the patients.

The yoghurt used contained $>10^8$ *Lactobacillus acidophilus* per ml. The lactobacilli showed "moderate production" of H_2O_2.

The primary efficacy variable was the number of infections on either study arm and this difference was significant: 0.38±0.51 in the active arm compared to 2,54±1.66. There was also a significant difference in favour of active treatment in the number of cultures positive for *Candida*: 0.84±0.90 and 3.23±2.17 respectively.

The study also reported a higher number of vaginal cultures positive for lactobacilli when there were simultaneously lactobacilli in stool. Whether this was the *Lactobacillus* given in the yoghurt is not stated.

One basic objection to this study is the lack of a placebo arm. *Shalev* and co-authors (1996) address this aspect in their study. The design was a blinded cross over study with one group of women eating 150 ml yoghurt with *L. acidophilus* daily for two months, followed by a two month wash out period and finally two months with pasteurised yoghurt. In the other arm the order was reversed.

The active treatment was a commercial yoghurt containing $>10^8$ CFU *L. acidophilus* per ml. It is uncertain whether any H_2O_2 producing capacity was verified.

The study included 46 women: 20 with BV, 18 with candidosis and eight women had both conditions simultaneously. Only seven women completed the full protocol and data are only given for the first four months of the study. At the visits after three and four months treatment only 22 and 17 women respectively attended. There are no data telling the initial diagnoses in these women.

With respect to *Candida* there was a reduction in the number of positive vaginal cultures in the first two months irrespective of treatment. 60% were culture positive at inclusion and this was reduced to 28% after two months. The

number of clinical recurrences was also unaffected by treatment. In the seven women who completed the protocol there were three episodes of vaginitis in 14 visits in the yoghurt period compared to five episodes in 14 control period visits.

TREATMENT OF BACTERIAL VAGINOSIS

The hallmark of BV is the absence of lactobacilli. Consequently the idea of treating the condition with lactobacilli is compelling.

In a letter in Lancet *Fredricsson* and co-workers (1987) reported on treatment of BV with four different modalities. They included 61 women with BV in an open randomised study, treating them for one week with two daily doses of respectively 5 ml 0.92% v/v acetic jelly (Aci-Jel®), 5 ml Dienoestrol® cream, 5 ml yoghurt with *L. acidophilus* or 500 mg metronidazole. Thirteen of the 14 women treated with metronidazole were cured at control after four weeks compared to only one of the 14 yoghurt treated.

Hallén and co-authors (1992) performed a double blind, placebo controlled study with local treatment of BV with lyophilised H_2O_2 producing *L. acidophilus* of human origin (Vivag®, Pharma-Vinci A/S, Denmark). The vaginal capsules contained 10^8 to 10^9 CFU of lactobacilli. Treatment was with one capsule twice daily for six days. Sixty women with BV were included; there were three dropouts. The first control was immediately after treatment when 12 out of 28 (43%) actively treated women had normal findings compared to none of the placebo treated. Protocol design indicated treatment with metronidazole to patients with persistent BV so no relevant comparisons can be done from visit number two, performed after the next menstrual period. However 14 of the actively treated returned and 11 of these had relapsed into BV. Of the Vivag® treated women, 93% had *Bacteroides* at inclusion and this was reduced to 35% after treatment. Whether the lactobacilli in the vagina were the strain from the vaginal capsules was not investigated.

Neri et al (1993) compared *L. acidophilus* with acetic acid in the treatment of BV in 64 pregnant women. This was an open randomised study and the women were given two daily doses for seven days of either 10-15 ml of yoghurt with *L. acidophilus* (>10^8 CFU/ml) or a vaginal tampon soaked with 10-15 ml of 5% acetic acid. The yoghurt was introduced into the vagina with a syringe. There was also a control group of 20 women who refused treatment of their BV.

Outcome was significantly better for women treated with yoghurt compared to both acetic acid and no treatment. Twenty-eight of yoghurt treated women were free from BV one and two months after treatment compared to 12 at each visit in the acetic acid group and three and one in the control group.

In a placebo-controlled, randomised three-centre study *Parent* and co-workers (1996) investigated a vaginal tablet containing H_2O_2 producing *L. acidophilus* and 0.03 mg estriol (Gynoflor®, Medinova Ltd). They included 17 women in the active group (six pregnant) and 15 in the placebo group (2 pregnant). There were two different dosings in each group, one or two vaginal tablets daily. Since there were no differences in the outcome measures irrespective of treatment data were pooled. Of 32 included 25 returned for the first control after one

week and only 17 to the control after three weeks.

Active treatment was significantly better: 77% were free from BV at the first control and 88% at the second compared to 25% and 22% respectively in the control group.

The study by *Shalev* et al (1996) mentioned earlier also included a number of women with BV, although the outcome in the BV group is not reported separately. However, 20 women with BV were included and eight with concomitant BV and candidosis. During active treatment the percentage of women with BV was significantly reduced from 55% at inclusion to 25% after one month and only 10% after two months. In the placebo group the number decreased from 50% to 40%. In the seven patients completing the protocol there was only one episode of BV during active treatment compared to six of them having one or two BV episodes during the period with pasteurised yoghurt.

DISCUSSION

To the clinician the treatment of vaginal infections with lactobacilli is an area filled with questions. One basic problem is knowing the composition of the normal flora of lactobacilli in different ecological niches. And do these lactobacilli differ in aspects that are relevant to the clinician?

Information so far seems to indicate that treatment with lactobacilli might be useful in the treatment of candidosis and BV. However the reports published are weak evidence. Patient numbers are small, attrition rates often very high and it is often difficult to deduce from the reports what really happened with patients in different diagnostic groups. Furthermore lactobacilli used are poorly defined and it is never verified whether the lactobacilli used for treatment ever colonised the vagina.

There are also reports on different strains of lactobacilli producing antimicrobial substances. Whether these lactobacilli might be better candidates for treatment, possibly in different clinical situations, is still not investigated.

So we really do not know whether lactobacilli work. What strains should we use? For what diagnoses? Should we use them for treatment or for prophylaxis?

It is also necessary for the microbiologists to help the clinicians investigate and define products with lactobacilli. The paper by *Hughes* and *Hillier* (1990) demonstrated that commercial products might contain no lactobacilli whatsoever or be contaminated with other bacteria.

In my opinion clinicians need alternatives to the well-defined antimicrobials and antifungals we have access to. Particularly for patients with recurrent symptomatic BV and chronic candidosis we urgently need additional treatment options to be able to better address these difficult clinical situations. More investigations are needed to prove the position for lactobacilli in this area.

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CHLAMYDIAL INFECTIONS OF THE FEMALE UROGENITAL TRACT

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SUMMARY

Bacteria in the genus *Chlamydia* comprise four species, namely *C. trachomatis*, *C. psittaci*, *C. pecorum* and *C. pneumoniae*. *C. trachomatis* infection is common, varying in prevalence up to 37%, in women. In the United States and perhaps elsewhere the overall prevalence rate among women is estimated to be about 5%. Pregnancy may predispose to an increased chance of infection with *C. trachomatis* through physiological immunosuppression and/or cervical ectopy.

The cervix, not the vagina, is the primary target for *C. trachomatis* infection which is often asymptomatic. Urethritis frequently accompanies cervical infection, but the main complications come from spread to the upper genital tract which leads to endometritis, salpingitis, perihepatitis and periappendicitis. The eventual outcome may be ectopic pregnancy, tubal infertility and chronic pelvic pain. Much of the pathology seems to be mediated immunologically. Fibrosis and scarring are a feature and it is possible that cytokines, particularly those that stimulate fibroblast activity (interleukin I and tumour necrosis factor), participate in the pathogenesis.

In pregnancy, *C. trachomatis* has been associated with premature rupture of the membranes, stillbirth and low birth-weight infants. The incidence of vertical transmission of chlamydiae from mother to baby varies; if the mother is untreated, 20%-50% of the new-borns will develop conjunctivitis and 10%-20% will develop pneumonia.

C. psittaci infection in pregnancy is rare but it can cause abortion, particularly in women who come into contact with infected sheep during the lambing season.

Accurate diagnosis of *C. trachomatis* infection has improved considerably with the advent of molecular techniques for amplifying chlamydial DNA. This, together with the ability to detect *C. trachomatis* in urine and vaginal swabs, is proving helpful in the promotion of screening programmes. Tetracyclines, usually doxycycline, and erythromycin and azithromycin form the mainstay of treatment.

INTRODUCTION

Chlamydial infection has been recognised since antiquity in the form of conjunctival blindness. However, it is only relatively recently, at the turn of this century, that the genital manifestations of non-gonococcal infection were

recognised in some individuals as being chlamydial. Isolation of *Chlamydia trachomatis*, one of the four *Ghlamydia* species, was achieved initially by using the yolk sac of embryonated hens' eggs and in the 1960s by using cell cultures, which were more sensitive. However, the slow delivery of a result often rendered the procedure of limited clinical value. The development of antigen detection tests improved diagnosis somewhat, but not as much as the most recent tests based on DNA amplification.

C. trachomatis was and probably still is the most common cause of pelvic inflammatory disease in the western world, and the consequent high rate of tubal damage makes this microorganism the most common cause of tubal infertility. *C. trachomatis* infection has an incubation period of 10-14 days, but latent asymptomatic infection may last for months or even years. In this review chlamydial infections in women are highlighted and the effect on pregnancy and the new-born are discussed, as are laboratory diagnosis and approaches to treatment.

MICROBIOLOGICAL BACKGROUND

Taxonomically, chlamydiae are placed in their own order (Chlamydiales) and the family, Chlarnydiaceae, contains a single genus, *Chlamydia*, which comprises four species. (*Chlamydia trachomatis* contains 15 serovars; serovars A-C cause the chronic cicatrising eye disease, trachoma and, rarely, sexually related infection (*Mabey* and *Whittle*, 1982); serovars D-K do not seem to be associated with trachoma, but cause paratrachoma and a variety of genital tract diseases. Serovars L1-L3 are responsible for lymphogranuloma venereum (LGV). *C. psittaci* causes disease in birds and animals and respiratory disease (psittacosis) and occasionally abortion in humans. *C. pecorum* causes pneumonia, arthritis and diarrhoea in cattle and sheep. *C. pneumoniae* is the recent binomial designation for strains which hitherto were termed TWAR. It causes human respiratory disease and has been associated with artherosclerosis.

Chlamydiae are recognised as bacteria because they possess peptidoglycan cell wall material, contain RNA and DNA and are sensitive to a wide variety of broad-spectrum antibiotics. However, the size of the infectious particles (elementary bodies: EBs) is similar to that of the large viruses (300 nm) and, like viruses, they have an obligate intracellular existence. This is probably due to their inability to generate adenosine triphosphate and the need to acquire it from the host cell to drive their own metabolic processes. The intracellular reproductive cycle comprises several well recognised phases (*Ward*, 1988), that is attachment of the metabolically inactive EB to the host cell surface, presumably through specific receptors and adhesins, and phagocytosis; conversion to a metabolically active reticulate body (RB) after about 8 hours; increase in the number of RBs by binary fission (cytoplasmic 'inclusion' formed); reorganisation of RBs to new EBs (larger inclusion) after 24-30 hours and, finally, release of the infectious EBs from the cell.

Recognition of late inclusions by staining with vital dyes or immunocytological techniques forms the basis of chlamydial detection in cell culture. Inclusions produced by *C. trachomatis* contain glycogen and, therefore, stain with iodine whereas those of the other chlamydial species do not. The EBs of many, but not all, *C. pneumoniae* strains are pear-shaped and have large periplasmic spaces. This distinguishes

them, by electron microscopy, from those of the other species which are round and have barely discernible spaces.

EPIDEMIOLOGY

C. trachomatis genital tract infection is common; prevalence rates varying from 2% to 37% have been reported for asymptomatic women in the United states (*Hammerschlag* et al., 1979; *Hardy* et al., 1984). Studies of pregnant women have revealed prevalence rates of chlamydial infection similar to those for non-pregnant women. In general, in both pregnant and non-pregnant groups, higher prevalence rates are found among indigent populations in urban areas, but overall it has been estimated that the prevalence rate in the United States is about 5% (*Schachter* et al., 1986). Studies based on gynaecological clinics and general practice in the United Kingdom have provided prevalence rates ranging from 3% to 12% (*Ridgway* et al., 1983; *Smith* et al., 1991).

The main demographic factors associated with high *C. trachomatis* prevalence rates in non-pregnant women are young age (24 years or less), low socio-economic class, single marital status, use of oral contraceptives or no contraception, intercourse with a new partner in the preceding two months, and living in an urban area (*Handsfield* et al., 1986). Risk factors for chlamydial cervical infection occurring in pregnant women are similar but also include the presence of mucopurulent cervicitis, abacteriuric pyuria and late antenatal clinic booking (*Sweet* and *Gibbs*, 1990). It is not clear whether oral contraceptive usage prior to pregnancy affects the rate of infection with *C. trachomatis* in pregnancy.

C. trachomatis has been detected in up to about 50% of women with gonococcal infection attending sexually transmitted disease (STD) clinics. Chlamydial infection prevalence rates usually parallel those for gonococcal infection, but in most populations chlamydial genital infections are three to four times more common than gonorrhoea (*Eager* et al., 1985), a ratio which may be higher in pregnancy.

DISEASE IN WOMEN (Table 1)

Bartholin's gland abscess

It seems that purulent infection of Bartholin's ducts may be due to chlamydial infection (*Davies* et al., 1978), either alone or with concurrent gonococcal infection. However, apart from a single further report (*Saul* and *Grossman*, 1988) of chlamydiae occurring in an abscess, little has been done to establish a causal relation.

Cervicitis

The cervix would seem to be the primary target for *C. trachomatis* infection, but its removal by hysterectomy does not necessarily mean that chlamydiae will not be found in the vagina (*Barton* et al., 1985), although their ability to cause vaginitis in the adult is unlikely. Infection of the cervix by *C. trachomatis* is often asymptomatic (*Leclerc* et al., 1988; *Cates* and *Wasserheit*, 1991). *Rahm* et al. (1988) noted that one-sixth of asymptomatic infected adolescents developed symptoms within 3 months. Indeed, chlamydiae are well-known to cause mucopurulent/follicular cervicitis, a condition that has been ex-

Table 1: Assessmont of the extent to which *C. trachomatis* is involved in various oculogenital and associated diseases

Disease	Evidence that *C. trachomatis* is a cause*	Proportion of disease due to *C. trachomatis*
In women:		
Urethritis	+++	?
Bartholinitis	+	?
Vaginitis	-	
Bacterial vaginosis	-	
Cervicitis	++++	About 50%
Cervical dysplasia	+	
Endometritis	+++	?
Salpingitis	++++	40-60%
Periappendicitis	++	?
Perihepatitis	+++	?
Infertility	+++	≥8% due to chlamydial salpingitis
Ectopic pregnancy	+++	?
Abortion	+	
In men or women:		
Conjunctivitis	++++	?
Otitis media	++	?
Arthritis (Reiter's syndrome)	+++	About 40%
Endocarditis	++	?
Pharyngitis	-	
Proctitis	++	?
Lymphogranuloma venereum	++++	100% (by definition)
In infants:		
Conjunctivitis	++++	Up to 50%
Pneumonia	++++	30%?
Chronic lung disease	++	?
Gastroenteritis	-	

*: ++++ = overwhelming; +++ = good; ++ = moderate; + = weak; - = none

amined in detail (*Dunlop* et al., 1989).Nevertheless, it is not clear how common chlamydial cervical infection is because the proportion of asymptomatic infections is unknown. It is assumed that many are, but this notion may be warped because studies have involved predominantly STD clinic populations where most chlamydial infections are detected in sexual contacts of men with non-gonococcal urethritis or gonorrhoea, that is in women who have attended the clinic because of their contact history and not because of symptoms. Asymptomatic infections may abound in the community at large, but it is noteworthy that the majority of infected women in a general practice setting had symptoms (*Longhurst* et al., 1987). Factors influencing the initial acquisition of chlamydiae are numerous and must include age, frequency of exposure, use of contraceptives, the role of which has been debated (*Edelman*, 1988), and

spermicides (*Ehret* and *Judson*, 1988). In addition, the hormonal status and the presence of ectopy and host defences influence acquisition. The mix is so complex and doubtless variable that in an individual case it would probably be unrewarding and perhaps impossible to determine the relative contribution of each, although certain risk-factors have been outlined (*Magder* et al., 1988). Although postmenopausal chlamydial cervicitis has been purported to occur (*Nagashima*, 1987), patients under 25 years old, those who use oral contraceptives and those who have signs of cervicitis are more likely to have a chlamydial infection, although these factors are not always predictive (*Kent* et al., 1988). One would also like to think that host defences, in terms of both cell-mediated immunity and pre-existing antibody, have an important role in determining acquisition because it may be possible to enhance any protective influence they have. Of course, knowing who is most likely to be infected based on a multiplicity of historical and clinical characteristics is helpful in deciding who should be screened. Selection may be required because routine testing may only be cost-effective if the prevalence of chlamydial infection is relatively high (*Phillips* et al., 1987).

Meijer et al. (1989) found a correlation between *C. trachomatis* infection and inflammatory, but not neoplastic, changes of cervical cells. However, *Paavonen* et al. (1998) have provided serological evidence that *C. trachomatis* infection increases the risk of subsequent development of invasive squamous cell carcinoma of the uterine cervix.

Women with chlamydial cervicitis often have an associated and frequently asymptomatic urethritis (*Horner* et al., 1995) but the main complications of cervical infection derive from spread of chlamydiae to the upper genital tract.

Pelvic inflammatory disease (PID)

Canalicular spread of chlamydiae to the upper genital tract, which may be completely asymptomatic (*Stacey* et al., 1990; *Tait* et al., 1997), leads to endometritis, often plasma-cell associated (*Paavonen* et al., 1985) and sometimes intensely lymphoid in reaction (*Thomas*, 1986). Further spread causes salpingitis, perihepatitis, sometimes confused with acute cholecystitis in young women (*Shanahan* et al., 1988), in addition to peri-appendicitis and other abdominal complaints (*Duffy* et al., 1985), although the organisms are not always found in the cervix when these conditions are recognised (*Moller* et al., 1986). Factors contributing to the spread of the organisms are the time in the menstrual cycle during which acquisition occurs, serovar and infecting dose, duration of infection, presence of associated infections, absence of antibody, hormonal status and also the integrity of the genital tract. The most important of these in an individual case may be impossible to identify, although the trauma of surgery, for example termination of pregnancy (*Heisterberg* et al., 1985), or insertion or removal of an intrauterine contraceptive device, are obvious predisposing factors. So too is chorionic villus sampling, but routine screening before sampling is not cost-effective unless the procedure is being undertaken in a high-risk population (*Moncada* et al., 1987).

Chlamydial infection has been the major cause of salpingitis in Scandinavia (*Mårdh*, 1986) but it is unknown exactly how common chlamydial salpingitis is in most countries, because laparoscopy required for clinical diagnosis and for obtaining specimens to distinguish accurately between upper and lower genital tract infection is not undertaken routinely. Whether it will be possible to undertake laparoscopy more often and/or develop non-invasive pro-

cedures of sufficient sensitivity and specificity to be helpful still remains to be seen.

The eventual outcome of PID may be tubal infertility, for which there is direct isolation evidence (*Brunham* et al., 1988) and indirect serological evidence (*Robertson* et al., 1987) to link it with chlamydial infection; it seems that this often may be asymptomatic (*Cates* et al., 1994). Other consequences are ectopic pregnancy, which also may arise as a result of a subclinical chlamydial tubal infection and for which a serological association with chlamydiae has been seen (*Cates* and *Wasserheit*, 1991), and chronic pelvic pain. What factors determine precisely the development of such sequelae in chlamydial PID are unclear, although there is evidence from the Scandinavian studies that the number and severity of the infections influence subsequent fertility rates (*Westrom* et al., 1992). Infertility could be due to endometritis, or blocked or damaged tubes resulting from cellular infiltrates, or perhaps abnormalities of ovum transportation, as suggested by the results of work on a mouse model (*Tuffrey* et al., 1986).

THE EFFECT OF PREGNANCY ON *CHLAMYDIA TRACHOMATIS* INFECTIONS

Rates of isolation of *C. trachomatis* from the cervix have been reported to be higher in the second and third trimester than in the first (*Brunham* et al., 1990). However, it is not known whether pregnancy increases the degree of shedding of chlamydiae from the cervix, because the data were derived from patients who were examined only at antenatal booking and may merely reflect the increased risk of *C. trachomatis* infection in those booking in the third trimester. Nevertheless, chlamydial infection is associated with cervical ectopy, a physiological condition predisposed to by an increased serum concentration of oestrogen, and it is plausible that the ectopy associated with pregnancy predisposes to increased shedding of, and/or risk of infection with *C. trachomatis*.

Pregnancy is physiologically immunosuppressive and cell-mediated immune responses are reduced progressively with advancing gestation to a nadir at 32 weeks gestation, recovery occurring by term in the absence of any superimposed immunosuppression. However, whether this suppression affects replication and shedding of *C. trachomatis* is unknown.

Maternal IgG antibody produced in response to *C. trachomatis* infection begins to cross the placenta after 5-6 weeks of gestation and is transferred at a more or less constant rate up to 17 weeks of gestation, after which there is an increase with increasing gestational age. However, up to two-thirds of babies born to mothers with genital *C. trachomatis* infection become infected, so that this passive maternofetal immunisation is, if at all, only partially protective. Specific antibodies are found also in breast milk, but the protective value to the new-born is unknown (*Brunham* et al., 1990).

THE EFFECT OF *CHLAMYDIA TRACHOMATIS* ON PREGNANCY

Infection with *C. trachomatis* can occur at any time throughout pregnancy and in the postpartum period; the manifestations of infection depend on the trimester in which it occurs.

Infection in the first trimester of pregnancy

Surgical termination of pregnancy in a woman with chlamydial cervicitis may cause PID with endometritis and/or salpingitis. Approximately 20% of patients with *C. trachomatis* infection prior to termination have developed salpingitis (*Giertz* et al., 1987). Many authors have advocated screening for *C. trachomatis* to avoid this iatrogenically induced pathology. Prophylactic use of erythromycin has been shown to reduce the incidence of postabortal PID, but it is debatable whether prophylaxis in the absence of screening is of value (*Sorensen* et al., 1992).

It is not known whether infection with *C. trachomatis* predisposes to spontaneous abortion, since the high background rate of both *C. trachomatis* and spontaneous abortion makes causation difficult to either prove or refute.

Infection in the second and third trimesters of pregnancy

Chorioamnionitis, premature labour and rupture of the membranes have all been associated with *C. trachomatis* infection, but whether the infection is a cause needs to be looked at more closely. Early prospective studies in pregnant women failed to show an association of chlamydial infection with prematurity (*Schachter* et al., 1979), but most of the women in these studies were enrolled in the third trimester; when enrolment occurred earlier, at 19 weeks of gestation, a highly significant association was found between infection with *C. trachomatis* and stillbirth, premature birth and perinatal death from prematurity (*Martin* et al., 1982). In another study, *Harrison* et al. (1983) showed no association overall between *C. trachomatis* infection and spontaneous abortion, stillbirth, premature labour or rupture of membranes. However, in a subgroup of women in whom *C. trachomatis* infection was detected by culture and by having IgM chlamydial antibody (24% of those infected with *C. trachomatis*), the infection was associated with low birth-weight infants and with premature rupture of membranes. This led to the hypothesis that recent exposure to *C. trachomatis* was important in its pathogenic effect on the chorioamnion.

Postpartum infection

The existence of postpartum chlamydial endometritis in mothers of children born with inclusion conjunctivitis was confirmed in a prospective study by *Rees* et al. (1977). Endometritis may be asymptomatic or patients may present with secondary postpartum haemorrhage, fever, lower abdominal and/or vaginal discharge. Postpartum endometritis may be subdivided into early (within the first 48 hours after birth) or late (3 days to 6 weeks after birth). Chlamydial postpartum endometritis tends to fall into the late category and usually develops 2 to 6 weeks after birth. Late postpartum endometritis occurred in 22% of women with antepartum *C. trachomatis* infection and in 5% of those who were uninfected (*Wager* et al., 1980) and tended to be associated with vaginal delivery. In another study of women with late postpartum endometritis after vaginal delivery (*Hoyme* et al., 1986), 23% had *C. trachomatis* detected in the endometrium and a further 37% had the microorganism detectable in the cervix, but the majority of patients were a-febrile and not seriously ill.

THE EFFECT OF *CHLAMYDIA PSITTACI* ON PREGNANCY

This review highlights *C. trachomatis*, but it is necessary to consider *C. psittaci* because of its known effects on pregnancy. There is no evidence for any

effect of *C. pneumoniae* on pregnancy, but *C. psittaci* organisms may be transmitted from various birds and mammals to humans and such infections occasionally command attention. Thus, it is known that in the United Kingdom and France, pregnant women have aborted after exposure to *Chlamydia*-infected sheep during the lambing season (*Giroud* et al., 1956; *McKinlay* et al., 1985). *C. psittaci* strains of ovine origin have been isolated from placental samples of women, usually sheep farmers' wives, who have been in contact with aborting ewes. The women also exhibited antibody responses.

Although the exact mechanism of placental involvement and abortion is unknown, the pathological features suggest the likely course of events. *C. psittaci*, acquired presumably through the respiratory rather than the genital route, escape into the maternal circulation and invade the placenta because of a predilection for the human trophoblast. There they multiply rapidly, are released into the intervillous spaces and spread to other chorionic villi, inducing an intense acute inflammatory response. Free EBs in the intervillous spaces, augmented by others released from degenerating trophoblast tissue, are phagocytised by inflammatory cells. while those in the trophoblast invade deeper into the placenta, producing a foetal stem vasculitis. The considerable tissue damage causes placental insufficiency and foetal anoxic death. The maternal disseminated intravascular coagulation/shock syndrome is probably due to the destruction of trophoblast tissue, releasing large amounts of thromboplastic materal and/or chlamydial endotoxin into the maternal circulation.

Studies of the prevalence of *C. psittaci* antibodies in sera collected from workers on farms in northern England where chlamydial ovine abortion occurred (*Hobson* and *Morgan-Capner*, 1988) indicated that human infection with ovine *C. psittaci* strains was uncommon. Antibody was detected no more frequently in farmers and their wives than in the non-farming adult community. Indeed, as indicated above, only a few cases of human abortion arising in the way described have been recorded. Nevertheless, it is clearly prudent to advise pregnant women to avoid contact with sheep, especially in the lambing season.

THE EFFECT OF *CHLAMYDIA TRACHOMATIS* ON THE NEW-BORN

Chlamydial infection of infants delivered by caesarian section and/or those who have signs at birth (*Attenburrow* and *Baker*, 1985) indicates that intrauterine infection can occur. However, the major risk to the infant of acquiring a chlamydial infection, which may manifest as conjunctivitis and/or pneumonia, is from passing through an infected cervix. Whether or not chlamydial infections of the new-born constitute a problem will depend on the prevalence rate of cervical infection which, as indicated previously, varies widely.

Conjunctivitis

Various studies have shown that between one-fifth and one-half of infants exposed to *C. trachomatis* infecting the cervix will develop conjunctivitis. The disease occurs usually from 5 to 19 days after birth and is characterised by a mucopurulent discharge and occasionally by pseudomembrane formation. Although it might be quite severe, corneal ulceration and follicle formation are rare and the disease is usually self-limited and resolution occurs without visual impairment; if complications

arise, they tend to be in infants that have not been treated.

Respiratory tract infection

The realisation that *C. trachomatis* could cause neonatal pneumonia lagged behind its recognition as a cause of conjunctivitis. The association with pneumonia was brought into focus by *Beem* and *Saxon* (1977) who described a series of cases. Overall, about 10-20% of exposed infants develop pneumonia (*Schachter*, 1988), that is about half of those that develop conjunctivitis. However, pneumonia is not always preceded by conjunctivitis. Chlamydial pneumonia occurs usually between the fourth and eleventh weeks of life, preceded by upper respiratory symptoms. A history of recent conjunctivitis and bulging eardrums is found in about half the cases. The disease is characterised by an a-febrile protracted course in which there is tachypnoea and a prominent stacatto-type cough. Generalised hyperinflation with bilateral, diffuse and symmetrical interstitial infiltration with scattered areas of atelectasis are the radiographic findings.

The exact way in which pneumonia develops is unknown, although a relative eosinophilia in some cases has suggested the possibility of a hypersensitivity mechanism. However, whatever the mechanism, there is evidence that the disease can lead to permanent lung damage. Thus, children who have experienced chlamydial infection during infancy are more likely to develop obstructive lung disease and asthma than are those who have had pneumonia due to other causes or healthy controls (*Weiss* et al., 1986).

INFORMATION FROM ANIMAL MODELS

It is easier to draw conclusions about the ability of chlamydiae to cause human disease if the animal model is a subhuman primate. The more distant the phylogenetic relationship, the more difficult it is to make inferences. Despite this, small animal models have had and still have a lot to offer, particularly in relation to mechanisms of pathogenicity. For example, because of their short reproductive cycle, mice provide an excellent model for investigating mechanisms of *C. trachomatis* induced infertility (*Tuffrey* et al., 1986). Such infertility appears to be due to failure in transportation of ova to the oviduct even when the tubes are not occluded. This could account for ectopic pregnancies associated with chlamydial infection in women. The mouse model has been used also to investigate the effects of *C. trachomatis* infection on pregnancy outcome (*Tuffrey* et al., l987). When mice were inoculated intraperitoneally, or both intravenously and intravaginally, chlamydiae were isolated from at least one placental disc in about a quarter of the mice, but never from foetal tissue even when there was heavy placental colonisation. Thus, unlike *C. psittaci*, *C. trachomatis* did not cross the placenta. This is consistent with the fact that the pregnancy outcome in these mice was unaffected (*Gale* et al., 1986).

PATHOGENESIS AND IMMUNE RESPONSE

The immune response to chlamydial infections may be protective or damaging, and contribute to the pathogenesis of disease (*Monnickendam*, 1988; *Taylor-Robinson* and *Ward*, 1989; *Witkin*, 1995).The hallmark of

chlamydial infection, whatever the anatomical site, is the lymphoid follicle. Follicles contain typical germinal centres consisting predominantly of B lymphocytes, with T cells, mostly CD8 cells, in the parafollicular region. Between follicles the inflammatory infiltrate contains plasma cells, dendritic cells, macrophages, and polymorphonuclear leukocytes, in addition to T and B lymphocytes. The late stage of chlamydial infection is characterised by fibrosis, seen typically in trachoma and PID. T lymphocytes are also present and outnumber B cells and macrophages. Biopsies taken from patients with cicatricial trachoma and persisting inflammatory changes show a predominance of CD4 cells, but those from patients in whom inflammation has subsided contain mainly CD8 cells.

Experiments using oviduct organ cultures suggest that direct cell damage is unlikely to account for chlamydial pathology. However, in view of the histopathological features that have been mentioned, the fact that chlamydiae cause immense damage to oviducts in the intact host and that vaccination has sometimes caused more damage than protection, it is reasonable to suppose that much of the pathology might be mediated immunologically. Longitudinal studies of trachoma have shown that certain individuals appear predisposed to persistent severe inflammatory disease, perhaps reflecting genetically determined differences in the immune response to sensitising chlamydial antigens. Evidence for the existence of sensitising chlamydial antigens is seen from the fact that repeated ocular infection by chlamydiae induces progressively worse disease with a diminished ability to isolate the organisms, features noted both naturally and experimentally. Also, primary inoculation of the oviducts of pig-tailed macaques with *C. trachomatis* has produced a self-limiting salpingitis with minimal damage, whereas repeated tubal inoculation has caused hydrosalpinx formation and adnexal adhesions. An exaggerated inflammatory response has also been induced by the ocular instillation of Triton X- 100 extract of surface antigens of the guinea pig inclusion conjunctivitis agent in previously infected, but not in naive, guinea pigs, the time course and histopathology of the response showing it to be due to delayed hypersensitivity. In a cynomolgus monkey model, a similar phenomenon was effected by a genus-specific protein of 57 kDa that has sequence homology with the GroEL heat-shock protein of *Escherichia coli*. However, while there seems no doubt about the importance of this antigen, its exact role in chronic non-gonococcal urethritis and PID is unknown. So too is the pathogenesis of fibrosis or scarring which occurs as a late sequel of chlamydial infection, typically in trachoma and PID. It is possible that interferon-γ may be responsible and feasible that other cytokines, particularly those that stimulate fibroblast activity, such as interleukin I and tumour necrosis factor-β, may participate in the pathogenesis of scarring.

LABORATORY DIAGNOSIS

The diagnosis of chlamydial infection, reviewed in detail recently (*Taylor-Robinson*, 1997), depends on detection of organisms or their antigens or DNA and to a much lesser extent on serology. It is worth emphasising that male and female 'first-catch' urine specimens, ignored for years because they were not suitable for chlamydial culture, are valuable samples, as are vaginal swabs, provided that the centrifuged deposits are tested by molecular methods.

Detection methods

Culture of *C. trachomatis* involves the centrifugation of specimens (not required for *C. psittaci*) usually on to cycloheximide-treated McCoy cell monolayers, and less often on to HeLa 229 cells treated with diethylaminoethyl (DEAE)-dextran. LGV strains are more likely to grow in cells that have not been treated with DEAE-dextran than are other *C. trachomatis* serovars. Isolation of *C. pneumoniae* is particularly difficult and may be facilitated by using a line of human lung cells. Inoculation of any of the cell cultures is followed by incubation and staining with fluorescent monoclonal antibody or with a vital dye, usually Giemsa, to detect inclusions; one blind passage may increase sensitivity. However, culture for *C. trachomatis*, despite its force in cases of litigation, is not practised often because it lacks sensitivity and is labour intensive and slow. The latter is not a feature of direct staining of specimens with species-specific fluorescent monoclonal antibodies, a technique that in competent hands allows detection of a few elementary bodies, even one. The method is most suited to laboratories dealing with a small number of specimens and for confirming positive results obtained by other tests.

The popularity of enzyme immunoassays that detect chlamydial antigens is due to their ease of use, but it is rarely possible to detect small numbers of organisms (<10) of whatever chlamydial species. Thus, since at least 30% of genital specimens contain such small numbers, many *Chlamydia*-positive patients are misdiagnosed. However, molecular techniques, that is those involving polymerase chain and ligase chain reactions, by enabling enormous amplification of a DNA sequence specific to the chlamydial species, have overcome the problem of poor sensitivity. Unquestionably, they have a place in research and in routine diagnosis and their existence is helping to promote screening programmes (*Boag* and *Kelly*, 1998).

Serological tests

A good correlation has been found between IgG and/or IgA antibody, measured by micro-immunofluorescence, in tears and the isolation of *C. trachomatis* from the conjunctivae of subjects with endemic trachoma or adult ocular paratrachoma. In genital infections, serum antibodies occur frequently in the absence of a current chlamydial infection of the cervix so that reliance cannot be put on a single serum or local IgA specific antibody titre to denote a current infection. In PID, pre-existing antibody or a delay in clinical diagnosis usually prevents confirmation on the basis of a rising antibody titre, but titres tend to be higher, especially in the Curtis Fitz-Hugh syndrome, than in uncomplicated cervical infections. A very high IgG antibody titre, for example 1:512 or greater, is suggestive of an aetiological association in pelvic disease, but high levels do not always correlate with detection of chlamydiae and are associated more with chronic or recurrent disease. In distinct contrast, the detection of specific *C. trachomatis* IgM antibody in babies with pneumonia is pathognomonic of *Chlamydia*-induced disease.

TREATMENT OF CHLAMYDIAL INFECTIONS

Up-to-date guidelines for the treatment of sexually transmitted diseases, including *Chlamydia*-induced disease, have been published recently (MMWR, 1998). Chlamydiae are particularly sensitive to drugs that interfere with protein synthesis, for example tetracyclines and macrolides, but are sensitive

Table 2: Susceptibility of *Chlamydia trachomatis* to various antibiotics*

Antibiotic	Minimum inhibitory concentration (MIC) (μg/ml)	Minimum bactericidal concentration (μg/ml)
Rifampicin	0.005 -0.25	0.015 - 0.25
Rosaramicin	0.015 -0.25	0.05 - 0.25
Minocycline	0.015 - 0.5	
Tetracycline	0.02 - 0.5	0.02 - 2.0
Doxycycline	0.025 - 0.5	
Oxytetracycline	0.03 -0.25	0.5
Erythromycin	0.03 - 0.5	0.1 - 4.0
Josamycin	0.03	
Roxithromycin	0.03	0.06
Miocamycin	0.06 -0.125	
Chlortetracycline	0.125 - 2.5	0.125 - 2.5
Azithromycin	0.125	
Clindamycin	0.25 - 2.0	
Spiramycin	0.5	
Ofloxacin	0.5 - 1.0	0.5 - 1.0
Ciprofloxacin	1.0 - 2.0	1.0 - 2.0
Benzylpenicillin	0.25 - 50	1.0 - >100
Ampicillin	0.25 - 50	>100
Sulphamethoxazole	0.5 - 50	
Chloramphenicol	1.0 - 10	>8 - 10
Augmentin	2.0	
Lomefloxacin	2.0 - 4	
Amoxycillin	2.0 - >4	
Rosoxacin	4 - 8	4 - 8
Sulphisoxazole	2.0 - 200	2.0 - 500

*: In addition, the following antibiotics with MIC values of >8 μg/ml have been tested and are shown more or less in order of increasing MIC value: amifloxacin, enoxacin, pefloxacin, trospectomycin, sulphamethiazole, cloxacillin, norfloxacin, cephaloridine, trimethoprim, spectinomycin, flumequine, novobiocin, nalidixic acid, kanamycin, lincomycin, colistin, gentamicin, vancomycin, metronidazole, streptomycin.

also to other drugs. The minimum inhibitory concentrations of a wide range of antibiotics are presented in Table 2 in which the antibiotics are listed in order of diminishing *in vitro* activity. Antibiotics with a minimum inhibitory concentration of 2.0 or more μg/ml are of no therapeutic value. The exact order in which the antibiotics are placed is arguable but the overall pattern is likely to be correct. The rifampicins are probably more active than the tetracyclines *in vitro* but are reserved usually for mycobacterial infections. Tetracyclines remain the drugs given most widely for chlamydial infections and resistance, if it occurs, does not seem to have accounted for the occasional anecdotal report of failure to respond to tetracyclines. Nevertheless, vigilance should be kept for tetracycline-resistant strains that could jeopardise clinical practice, as the use of non-cultural diagnostic procedures has made their detection less easy. Of the macrolides, erythromycin is used most often and is chosen for chlamydial infections in infants, young children and pregnant and lactating women. Azithromycin in a single dose remains expensive but is gaining favour

because it is effective and enhances compliance. Alternatives, such as some of the quinolones, particularly ofloxacin, are effective but have not found regular use.

In complicated genital-tract infections, such as PID, treatment will almost certainly be needed before a microbiological diagnosis can be established, following which additional broad-spectrum antibiotic cover may be required. In the case of *C. pneumoniae* and *C. psittaci* infections, treatment follows the same principles as for *C. trachomatis* infections, as they are susceptible to the same types of antibiotic. Finally, treatment is likely to be most effective when given over a long rather than a short time, sub-optimal doses are avoided, compliance is strict, and in the case of genital tract infections, partners of patients are also treated.

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OLD HERBORN UNIVERSITY SEMINAR ON THE VAGINAL FLORA IN HEALTH AND DISEASE: MINUTES AND REVIEW OF THE DISCUSSION

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The day and one-half period of discussion among the invited speakers touched on a variety of subjects which did not always proceed in an organised fashion. At times a subject was revisited from a slightly different perspective. Thus, for ease of reading, the discussion points are not reported in a chronological fashion but are organised according to topic as far as possible.

The discussion opened with a focus on urinary tract infection (UTI). Hooton questioned whether giving lactobacilli would be helpful in addressing recurrent UTI which has been observed in women aged 14 to 68 in Seattle studies. It was agreed that we are lacking knowledge about the value of such therapy in the young, prepubertal, and the postmenopausal. Hooton reported that knowledge of the incidence of UTI in postmenopausal women, with or without hormone replacement therapy (HRT) is not known or even under study. Some data are coming from Seattle, in studies that are currently underway, regarding lactobacilli and UTI in women aged 50-70.

Data do exist regarding bacterial vaginosis (BV) in postmenopausal women. There is a significant incidence of BV in postmenopausal women and it is known that lactobacilli populations are highly variable in postmenopausal women and is not related to age of the individual. The relationship of numbers of lactobacilli to glycogen content of the vaginal epithelium is not firm (Cauci).

British workers are studying postmenopausal women in an STD clinic as well as younger women in a clinic for lesbians. In the latter, approximately 50% are presenting with BV while in the former, low numbers of lactobacilli are observed and a higher incidence of BV than would be expected (Taylor-Robinson).

Discussion ensued regarding the clinical and laboratory basis for a BV diagnosis. There was agreement that elevated numbers of *Gardnerella vaginalis*, a diminished number of lactobacilli, an increase in anaerobes, a pH of greater than 4.5, the presence of clue

cells, a positive "whiff" test, and a Nugent score of 5-6 was consistent with BV. These assessments are based on the Gram-stain of a vaginal swab and are quite subjective; it was felt that a more quantitative approach is desired in order to better standardise BV diagnosis among different laboratories and clinics.

Taylor-Robinson described a longitudinal study of BV in women, based upon vaginal swab slides, in which the participants were going along fine and then observed a sudden fluctuation in bacterial numbers. This change tended to correlate with the early part of the cycle when the menstrual flow was heaviest. Carter asked if there might be growth factors for *G. vaginalis* in the sloughed endometrium similar to erythritol in the mammalian placenta which is a growth factor for brucellae. This remains to be determined.

The discussion moved to biofilms, described by Reid and others referring to William Costerton's work, in which a community of different microbes exist in a matrix of protein and polysaccharide on mucosal and other surfaces, such as tampons, with the anaerobic flora predominating close to the bottom. De Buysscher led the discussants through a review of Freter's scheme for microbial homeostasis on mucosal surfaces in the intestine. This scheme is based on antibody selection of populations of bacteria expressing continually changing surface antigens: The bacteria initially present (Colony A) express antigens (Ag) 1,2 which grow and expand their populations. The antigens drain, in the case of the vagina, to the iliac lymph node where an immune response is initiated. The antibody produced against Ag 1,2 selects against the population of bacteria present in the vagina and induces that population to change by expressing slightly different Ags (1',2',3) creating Colony A'. These bacteria would grow and expand the concentration of Ags 1',2',3 in the vagina and lymph and a subsequent immune response would select for a new colony, B. Since it has been suggested that the amount of antibody present in the vaginal mucosa and the thickness of the mucosa varies with the time in the menstrual cycle, it may be that the shower of antigens from the vagina into the lymphatic drainage also varies with the cycle. In this case the stimulation of the draining nodes would rise and fall on a 28 day cycle in women between menarche and menopause. What happens in postmenopausal women would, presumably, be different. Cauci questioned whether the thickness of the vaginal mucosa, known to be thinner in postmenopausal women not on HRT, did in fact change with the time of the month. Hooton reported that vaginal biopsies of university-aged women, done as part of a study conducted in Seattle, did not show any variation in thickness with the time taken during the menstrual cycle. This contradicts the published information cited by De Buysscher that indicates high progesterone present after menses correlates with a thinner vaginal epithelium which is rich in IgG-containing cells. Taylor-Robinson showed data from experiments in London in which mice were given exogenous oestrogen. Such treatment increased the number of indigenous bacteria in the vagina of the mice and increased the susceptibility of the mice to vaginal infection with other microbes, such as mycoplasmas. It was noted that the vaginal specimens showed an increase in the number of sloughed epithelial cells, without evidence of neutrophils, and these cells were covered with bacteria, suggestive of clue cells in the human. Mattsby-Baltzer commented that oestrogen is known to increase the viscosity of vaginal mucus.

In discussing such exogenous affects on the flora, Taylor-Robinson commented that semen appears to affect vaginal flora composition, that semen

from different men can cause a change in a woman's flora, and asked if amoxicillin therapy would do the same and predispose to BV. Krohn said that Hillier's reports do not support such an affect.

Hooton mentioned that UTI in older women has been suggested to be related to inadequate bladder emptying (and the same for men). This is in the literature but there is little or no evidence supporting it; the data on UTI in postmenopausal women is for the most part lacking. Reid asked what protects young or elderly women from UTI? Hooton reported that higher concentrations of *E. coli* have been observed in women with recurrent UTI but the enigma is that you can find such high counts in women without UTI. This may relate to differences in virulence factors. Reid mentioned that mannose-resistant, low fimbriae strains are also found in UTI.

Cauci stated that *E. coli* produce haemolysins and toxins in addition to having attachment factors – is antibody to these present in vaginal fluids and, if so, does this antibody diminish with age as is typical of other immune responses in the elderly? De Buysscher drew on what is already established in animals where antibody to *E. coli* attachment factors is highly protective. He also commented that, although new immune responses are not so active in older animals, they have a larger "library" to draw upon than the younger animal or person. Mattsby-Baltzer stated that no correlation has been observed between UTI and *E. coli* haemolysin production, attachment factors, or serum resistance. Hooton looked at fimbriae and haemolysin production in UTI strains from patients with symptomatic UTI or asymptomatic bacturia and found no correlation; there was a correlation with strains from cases of pyelonephritis and the presence of P fimbriae. By convention, asymptomatic bacturia is $>10^5$/ml of urine from several samples over a period of days. Mattsby-Baltzer reported than in a Swedish study, symptomatic versus asymptomatic bacturia was correlated with the presence of K1 capsules in the *E. coli* strains isolated; so-called "high responders" presented with symptoms. De Buysscher asked if the *E. coli* from asymptomatic bacturia patients are coated with antibody since in animal models they are. This was not known. Hooton reported that ~60% of *E. coli* from pyelonephritis cases lack P fimbriae.

Cauci reported on her study of 800 premenopausal and 2,000 postmenopausal women not on HRT. In the latter group, no lactobacilli and no bacteria, generally, were observed on Gram-stain (estimate: >1,000/ml in order to observe) and that the incidence of BV was approximately half that of premenopausal women or postmenopausal women on HRT (related to change in sexual activity?). It was suggested that Hillier or Hill might have additional data on postmenopausal women.

Hill asked what factors affect vaginal health. What is the normal bacterial population and how is it partitioned in the sense of biofilms and vaginal site (introitus, vaginal vault, cervix)? How do spermicides, intercourse, tampons, and diet affect the population? It was agreed that current knowledge is soft and that better defined studies on larger numbers of women are necessary to fully answer these questions. Krohn commented that physician or self-collected vaginal specimens show that bacterial populations are fairly consistent for an individual over time but that there is variation among individuals. Reid asked if there is a change in flora during times of tampon use; over one hour or eight hours. It is known that tampons, as one biomaterial which has been studied, do not induce an anaerobic environment, such as has been associated with BV, if anything, just the opposite (Davis). But it is not known

whether tampons alter the vaginal flora. Reid commented that we know that in a study of stents, over 90% had biofilms on their surface. This opened a discussion of whether we need a new paradigm for quantifying bacteria. We already recognise that plate counts do not accurately assess the number of bacteria in a sample which is why we speak of colony-forming units (CFU) but do we need to find a method which takes into account the mixture of bacterial types found closely associated in biofilms? In referring to the Seattle studies of vaginal wall biopsies, Hooton questioned whether there is a total turnover of vaginal epithelium and its attendant biofilm. The consensus was that the epithelium turns over continually and thus the biofilm would not be disrupted. Reid mentioned that *Candida* is the major cause of diaper rashes and that the *Candida*-biofilm interface is an important area for research into understanding yeast invasion of the mucosa.

Carter raised the issue of how food may influence the vaginal flora, not just the intestinal flora, and may sensitise individuals to yeast antigens as Dr. Orian Truss and others have suggested. Reid asked if yeast antigens are found in the blood stream which might cause systemic effects and whether they might be detected. (Note: German workers have found whole yeast cells in the blood stream of people following ingestion.) McDonald suggested that an appropriate experiment would be to compare vegetarians and nonvegetarians in regard to their complaints of suggested *Candida*-related symptoms. Van der Waaij and Krohn both commented on the difficulty of doing dietary studies correctly and there was general concern about the controls used by Truss and others. Van der Waaij indicated that people in dietary studies could not be trusted to stick with the protocol without being virtually held in confinement. Krohn mentioned that groups working for years on the influence of diet on heart disease or cancer have established consensus protocols which are well-accepted for conducting dietary studies. Hard data is urgently needed in this regard since people are reacting to anecdotal reports and trying a variety of remedies, reported in the popular press, in attempts to find some resolution of their chronic/recurrent UTI or vaginal discharge.

Hooton asked whether probiotics in food or vaginal suppositories work better if the strains are peroxide producers. Reid suggested using a mixture of peroxide positive and negative strains and collect data on colonisation from human volunteers after per oral administration. Taylor-Robinson reported that in their hands, a peroxide-negative, bacteriocin-positive lactobacillus strain inhibited *Gardnerella*. Maes stated that probiotics have had broad use in food animals but not for vaginal problems. Reproductive tract problems in food animals are generally localised in the uterus without involvement of the vagina (although studies of vaginal microecology have not been published and vaginal discharge, without metritis is not pursued). The point was made that food animals vary from primates in that they mate only during oestrus and so may not be as susceptible to vaginal infections outside of pregnancy. Taylor-Robinson cited Hillier's report of the use of peroxide-positive *Lactobacillus acidophilus crispatus* in women: Administration of either 10^5 or 10^8 CFU results in vaginal colonisation for months at the level of 10^6 CFU. If the woman was already colonised with peroxide-producing lactobacilli, colonisation with *L. a. crispatus* was better than when the woman was not. Reid commented that relying on one strain was probably not the correct approach and that a cocktail would be better. Carter asked Heidt and van der Waaij to comment in this regard based upon their work in animals and immunodeficient patients. Van der Waaij

suggested looking at the immune response to the lactobacilli to determine which may be more persistent. When autologous probiotics were suggested, he cautioned against the "French kitchen" approach of a little bit of this and a little bit of that. We need hard data to make these decisions. McDonald mentioned that she is aware that. *L. a. crispatus* can colonise individuals for five years and this is not autologous. McDonald added the cautionary note of being careful not to introduce lysogenic phages, the plague of food manufacturers, which might destroy a person's natural flora of lactobacilli. Reid mentioned that exogenous lactobacilli will not displace *Candida, Proteus*, etc. which are already established in the vagina; antibiotic therapy must be given to deplete these organisms before *Lactobacillus* therapy can be successful.

Mattsby-Baltzer noted that BV is sometimes difficult to diagnose if the patient lies on the fringes of what is accepted, i.e. lacking one or more of the diagnostic features or having a vaginal pH not much above normal ranges. She asked if there might not be subgroups of BV which could be recognised if the diagnostic parameters were better characterised or standardised; should we culture all cases and emphasise a decrease in lactobacilli and an increase in anaerobes in the diagnosis? Currently, some studies report only the Nugent or Amsell score. McDonald mentioned that BV patients with *P. mobiluncus* present with an intense inflammatory response which may suggest we are dealing with a different disease. Krohn felt that it was time to go past the Nugent criteria, which represented an initial attempt at standardisation of clinical evaluation and help predict an uncommon outcome in large study populations, and determine which anaerobes are associated with which clinical presentations. The Amsell score is based upon odour, which is very subjective and pH (it is easy to determine pH 4.0 or 5.0 with litmus paper but pH 4.4 – 4.7 is a problem; thus if pH 4.5 is diagnostic, you have variability in the determination of this diagnostic criterion). The Nugent scoring presents a problem in the evaluation of intermediate flora. In clinical BV, Cauci felt the Nugent score was not all that dependable because bacteria other than *Gardnerella vaginalis* matter. She demonstrated this with data presented in the following table:

Lactobacilli	*G. vaginalis*	*P. mobilis*	Nugent Score
2* (2)	>30 (4)	-	6
5 (1)	>30 (4)	-	5
25	5	1	5
25	2	1	4

* bacteria seen / microscopic field

Taylor-Robinson stated that BV is certainly not homogeneous and that the lack of uniformity among laboratories, reported at the recent Aspen meeting, was astounding. The strongest association with preterm labour is in women with BV, especially those with BV plus *M. hominis*. He asked whether antibiotic therapy was known to affect preterm delivery. Mattsby-Baltzer asked whether BV during pregnancy might indicate upper tract infection. Only a subgroup, responded Krohn, who cited Hillier's finding of *Bacteroides* in the chorionic membranes of some BV women but not all. Cauci and Mattsby-Baltzer mentioned that BV was observed in about 20-30% of pregnant women (and even nonpregnant) in Italy and Sweden. Krohn mentioned that bacteria are found in the amniotic fluid of less than 1% of women not in labour.

Mattsby-Baltzer asked about the basis for recurrences in BV. Taylor-Robinson said there are two groups: Those clinically cured who then develop a new infection and those not really clinically cured who have a recurrence. Krohn estimates that there are 10-20% of women of child-bearing age in the USA who have BV at any one time (12% in the UK [Taylor-Robinson] and the same in Australia [McDonald]); approximately 80% of treated women are cured. Blacks have consistently higher prevalence of BV that is an apparent enigma since, in general, they douche quite a bit more than white women. Taylor-Robinson mentioned "difficult" cases, women who keep coming back, and back, and back. The approach has been to treat with metronidazole or clindamycin at intervals so that the *G. vaginalis* populations get lower and lower.

Taylor-Robinson asked if vaginal bacteria were able to infect cells like mycoplasmas do. Mattsby-Baltzer reported that *Prevotella* strains adhere closely to the cell membrane of HeLa cells, as is typical of mycoplasmas, but only a few appear to enter. McDonald mentioned in pregnancy, Gram-stains show 1+ to 2+ neutrophils. Taylor-Robinson maintained that a PMN response in BV is rare. Several discussants mentioned that after 41 weeks gestation, there are a lot of inflammatory cells invading the term placenta.

Hooton reported that a recent Medline literature review he did, reflected the pitifully low number of researchers world-wide addressing the problem of UTI (20-30). He made a free-hand drawing of the incidence of UTI in females throughout life which reflected the blip among the very young, low levels through childhood with increasing incidence in the adolescent and fertile woman to menopause, with the highest incidence being in the elderly woman. This latter may be related to elderly care facilities in which the cleanliness of the elderly person may not be as good, due to inability of the person to care properly for herself and inadequate staffing. Hooton felt that the data on UTI on the postmenopausal woman, below 70, was the softest and felt that more epidemiological assessments needed to be made in this age group. BV, on the other hand, peaks in the 20's and 30's and tapers off soon after menopause. The statistics are somewhat difficult to assess since a lot of UTI are just asymptomatic bacturias. 50, 60, 70% in institutionalised men and women and this is not really proven to be due to anatomical changes with age which lead to inadequate bladder emptying. Citing Stamey's work, Hooton mentioned that women who develop UTI are observed to have the causative organism in their vagina months prior to presenting with UTI. No genetic typing of the microbes has been done to confirm that it is the same organism; most studies in this area only use culture. The question was asked whether quantitation of the microorganisms should also be done. It was mentioned that heavy growth of *E.*

coli is associated with adverse pregnancy outcomes. A further question was asked if recurrent UTI are caused by the same risk factors as sporadic cases. It was admitted that there is very little data on premenarchal girls since pelvic exams and thorough work-ups are usually only done on such girls if raped or abused.

Hill returned to the need for data on normal flora saying that you cannot know the importance of the vaginal flora in disease if more isn't known about it in health. Very little work has been done on normal flora and its role in candidosis. With the availability of over-the-counter vaginal creams for the treatment of yeast infections, young women are using these at the first sign of itching. Hooton said these creams, themselves, are known to alter the lactobacilli in the flora and so their broadened and increased use may have a negative effect and predispose the user to subsequent vaginal infections. Krohn reported that, in a recent study, 20-25% of university-aged women had used Monistat® in the past five months and that a higher incidence of Group B streptococci were noted in these women; there has been no longitudinal study of vaginal floral changes after anti-fungal use.

Reid returned to BV maintaining that we need much better data on its incidence and prevalence. To do this, BV needs to be better defined. This was echoed by McDonald who felt that more research into the parameters defining BV was desperately needed. Cauci felt that the clinical diagnoses, when made, are correct but as many as 30% of cases were being missed. Hooton felt that the same problem with definition exists with UTI and the vaginal flora. UTI is diagnosed if the bacturia is $>10^5$/ml but in Seattle, experienced clinicians will go as low as 10^2/ml.

Carter raised discussion of the work of Truss and others suggesting a role for subclinical candidosis and diet causing local and systemic health problems in many women. Heidt mentioned that dietary influence is not known but in studies of bone marrow transplant patients, antibiotics and chemotherapeutics did cause the intestinal flora to change and were associated with an increase in *Candida* (this increase was not profound).

In response to comments by Carter and McDonald on the ability of ureaplasmas to produce disease, Taylor-Robinson said that ueaplasmas were strongly associated with chorioamnionitis but their role in spontaneous abortion is undetermined. He cited a 1M pound MRC study, based upon the assumption that *U.urealyticum* is a sole cause of disease. Maurice Shepard's work on non-gonnococcal urethritis in male Marines could be faulted by his lack of data on *Chlamydia* which might have been present. Also, as demonstrated by Japanese and British workers, the presence of *M. genitalium* can only be confidently assessed using PCR since this organism is so difficult to culture. This having been stated, it was admitted that *U. urealyticum*, in pure culture (10^5 intraurethrally), will cause NGU.

Cauci noted that cultivation of anaerobes is difficult and expensive and some clinical microbiology laboratories are reluctant to undertake it. She maintained that a woman can have increased numbers of bacteria but if the host is able to counteract them immunologically or with other defence mechanisms, there is no clinical problem. It was suggested that a survey of IgA-deficient women should be performed. Others felt that IgA-deficient individuals generally do well because they have compensatory local responses. A thorough assessment of local immune responses in the vagina needs to be pursued.

De Buysscher returned to a discussion of local immune responses. Cauci reported that vaginal histamine and IgE increased in candidosis so perhaps in-

gestion of yeasts or yeast products induces an enhanced reaction in persons who are presensitised.

The session ended with a comment about HIV infection. It is apparent that vaginal inflammation increases the risk of HIV infection in unprotected sex with infected partners. If BV, even without inflammation, also enhances risk of HIV infection, this would represent a significant at risk population.

Carter said that he is left with the conclusion that *Chlamydia* are the only recognised frank pathogens of the urogenital tract. Others felt that this was true to a certain extent and that appropriate animal models would be very helpful in advancing the field. Maes discussed the problems of using animals which only mated during oestrus. Swine are reasonable models for uterine infections but little else. However, he did admit that this view might be based on the fact that vaginal discharge is not associated with a drop in fecundity and therefore has not been important in the pork industry and thus not studied by swine veterinarians. A closer look at the vaginal flora and vaginal infections in swine may reveal useful information relevant to the human. It was agreed that rhesus monkeys may provide the best model for studies of human relevance because of menstruation and their sexual habits. Such animals could also be used in controlled dietary studies since they, like swine, have a digestive tract similar in many respects to the human.