

CHAPTER - I

INTRODUCTION

1.1 VAGINAL DRUG DELIVERY

1.1.1 Background

There are various routes for drug administration. Each route has its advantages and disadvantages. For example, drug through the parenteral route goes very fast in the systemic circulation. Also it avoids drug metabolism and degradation in the liver and intestine. But major drawback of this route is patient noncompliance and potential hazard during administration. Other common routes for drug administration are transdermal and oral route. Transdermal route can be used only for the administration of small lipophilic drugs while oral route causes first pass metabolism and also enzymatic degradation. The vaginal cavity is an important area of the reproductive tract and acts as favorable site for delivery of drug due to an avoidance of first pass metabolism, great permeation area, rich vascularization, and relatively low enzymatic activity (Bernkop S. and Hornof M, 2003). Many studies have demonstrated the superiority of vaginal over the oral routes in terms of striking minimization of general and gastrointestinal side effects.

From past few decades, drug delivery via vaginal route has been of the great interest for the pharmaceutical companies. In the recent year, research has been focused on vaginal drug delivery system as logic alternative to oral or parenteral administration of drugs, which are specifically used for the treatment of fertility control, hormonal replacement therapy, vaginal infections, sexual transmitted diseases (STDs) and other gynaecological conditions. In addition, the vaginal cavity is a potential for noninvasive, controlled transmucosal delivery of both local and systemic therapeutically active compounds (Benziger D.P. and Edelson J, 1983). A small survey conducted to evaluate the preferences of females with regards to the use of intravaginal medications showed positive market outlook for this mode of delivery (Woolfson *et al.* 2000).

1.1.2 Advantages

- ☞ Vaginal administration offers lower doses, steadier serum concentrations, less frequent administration and uninterrupted delivery of drug than oral or transdermal drug delivery.
- ☞ Arteries and veins form a dense network around the vagina, providing a rich blood supply. Therefore, the vagina is well suited for the rapid and steady uptake of hormones (Sitruk-ware R, 2005).
- ☞ Drugs administered via vagina are not subjected to first-pass metabolism and avoids gastrointestinal interferences with absorption of medication. This has been reflected by the greater bioavailability of misoprostol after vaginal administration compared with oral delivery (Zieman *et. al.*, 1997).

- ☞ It's a preferred route over oral route in the patients those who experiences nausea and vomiting. For example, use of bromocriptine intravaginally in the treatment of hyperprolactinoma in women who suffer from nausea and vomiting with oral administration (Varmesh M, 1988).
- ☞ The self-insertion and removal of dosage form is possible without need to visit the doctor or hospital and provide female controlled drug delivery.

1.1.3 Limitations

Despite all the above mentioned advantages, there are few factors which limit the use of vaginal administration. Self-cleansing mechanism of vaginal tract, low permeability of mucosal membrane (in case of some drugs) and physical removal from the site of deposition are few of them. Vaginal irritation and use of applicator for formulations administration can also results in patient noncompliance.

1.1.4 Vaginal Anatomy with Respect to Drug Delivery

The vagina is a tubular, 10 cm long fibromuscular tube comprised of three distinct layers including an outer adventitia layer, a middle muscularis layer and the internal mucosal layer (Paavonen J, 1983). The physiological structure of vaginal wall that consist of an epithelium, lamina propria, muscularis and adventitia is illustrated in Fig. 1.1

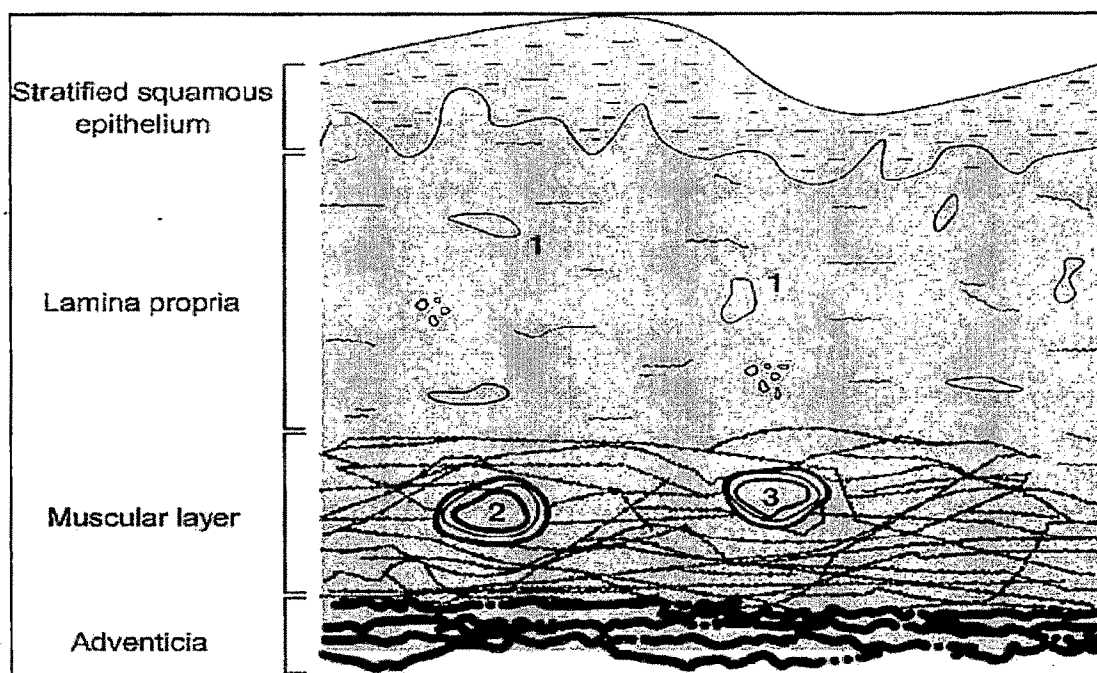
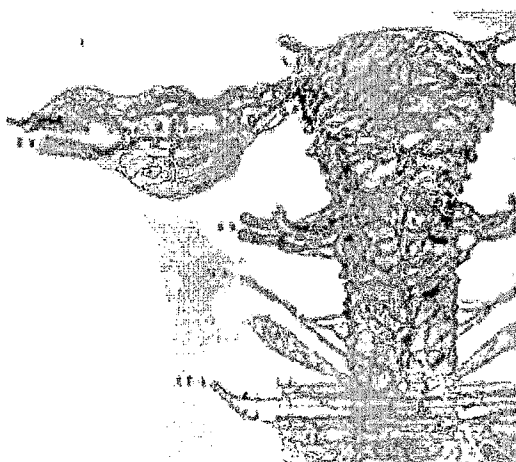


Fig. 1.1 Schematic drawing of the vaginal mucosa.
1: Capillary vessels; 2: Artery; 3: Vein

Epithelium of vaginal mucosa is consist of stratified, non-keratinized, glycogen containing squamous epithelium and areolar connective tissue. Epithelial thickness has been reported as 200 to 300 μm (reviewed by Woolfson *et al.*, 2000). Lamina propria is composed of dense connective tissue with numerous elastic fibers. Muscularis formed by bundle of smooth muscles and fibers that are continuous with those of myometrium of the uterus. Adventitia is the relative thin layer of dense connective tissues that contains many coarse elastic fibers.

Vaginal mucosa is rich in blood supply due to arteries and veins form a dense network around the vagina. Cicinelli E. *et al.*, (2004) reported that venous drainage drains into interior venacava by passing the hepatic portal system.



Arteries and veins form a plexus around the vagina,

Venous drainage of the vagina does not immediately pass through the liver,

These features make the vagina effective in the uptake of many drugs and stable for long-term, continuous drug delivery systems

The presence of larger amounts of phospholipids and loosely packed intercellular lipids in non-keratinized epithelium makes the vaginal mucosa more permeable than that of the skin stratum corneum (Robinson J.R., 1999).



The Vaginal Rugae,

- Increase the surface area of the vagina.
- Help to retain a formulation inside the vagina for longer time.
- Allow vaginal formulations to be placed in vagina with out injury.

The vaginal rugae and microridges on the epithelial cell surface permit the vagina to expand and allow the placement of a vaginal formulation. It also increases the surface area of the vagina and enhances drug absorption (Nelson A.L, 2004). It has remarkable features in terms of vaginal secretion, secretion pH, enzyme activity and microflora. These factors affect formulation spreading, retention, absorption and drug release in vagina.

1.1.5 Physiological Consideration

Vaginal Secretions: The vaginal epithelium has no goblet cell and lacks of the direct release of mucin. The labia minora at the entrance of the vagina have Bartholin gland and Skene's gland which produces mucous that aids in lubrication. Mucus is a complex aqueous mixture of glycoproteins; lipids, salts and cellular debris that may be adversely affect the absorption or action of drug administered by vaginal route (Nyririesy *et al.*, 1997). Many blood vessels are present in lamina propria that also exudates the lubrication fluid. The vaginal discharge is a mixture of multiple secretions that collect in the vagina from peritoneal, follicular tubal, uterine, cervical, Bartholin's and Skene's gland sources (Moghissi K.S., 1979). The rate of vaginal secretion is 1.55g/8hr; maximal at ovulation. Certain life events such as pregnancy, lactation, menopause or diseases will alter vaginal secretion. Some medicines include allergic, cardiovascular, psychiatric and other medical conditions will dry out the vaginal mucosa. Estrogens and sexual stimulation increase vaginal fluid secretion. However, the volume and viscosity of vaginal fluid may have impact on drug absorption. For example, the secretion of thick cervical mucus may present a barrier to drug absorption. Vaginal secretions have great concern in the development of solid formulations. In presence of moisture, formulations should ideally disperse in the vaginal cavity immediately after insertion to avoid inconvenience to the users and for rapid onset of activity.

Vaginal pH: Boskey E.R. *et al.*, (2001) reported that pH of the healthy female genital tract is acidic (pH 3.5–4.5) and is maintained by bacterial conversion of glycogen from exfoliated epithelial cells to lactic acid. Conversely, the male ejaculate is slightly alkaline with a pH of about 7.5 (Castle, P.E, 2002). For the successful delivery, the drug molecule must be stable over a wide range of pH. In addition, it should not have a negative impact on the acidic pH of the female genital tract. The pH change with age, menses, infections, estrogen deficiency and increasing level of cervical mucus. The control of vaginal pH is critical factor for successful vaginal delivery of ionizable drugs (Nyririesy *et al.*, 2001).

Enzyme Activity: Several enzymes includes, protease, β -glucuronidase, acid phosphatase, alkaline phosphatase, esterase, leucine aminopeptidase, aminopeptidase N, aminopeptidase A

and aminopeptidase B are found in vaginal cavity (menstrual cycle; life cycle). Acartürk F. *et al.*, (2001) reviewed the specific enzymatic activity of four different amino peptidases in all vaginal homogenates in the order: sheep > guinea-pig > rabbit > or = human > or = rat. The human genital tract has lower enzymatic activity than the gastrointestinal tract. Hence, vaginal route is a potential for protein delivery.

Cyclic Changes in Vaginal Epithelium: Guyton A.C. *et al.*, (1998) and Garg S. *et al.*, (2003) reported that physiologic changes in the vagina leads to variation in vaginal secretion, pH, enzyme activity, changes in the thickness and permeability of the epithelium during various phases of the menstrual cycle.

Vaginal Routes of Drug Absorption: The drug is delivered in vagina via mainly two routes, intravaginally to the vaginal epithelium or transvaginally through the vaginal mucosa to uterus and to the systemic circulation without need of being digested or processed (D'Augustine M.A, 2000). Richardson J.L. *et al.*, (1992a) reported that drugs are transported transvaginally by transcellular route, intracellular or vesicular and receptor mediated transport mechanism. Cicinelli E. *et al.*, (2000) reported that vagina has specific blood flow characteristics either by a portal type circulation or by venous and lymphatic channels. They permits transport and delivery of drug molecules from the vagina to the uterus and to the systemic circulation. The cyclic changes in vaginal epithelium and physicochemical properties of therapeutic molecules may alter the degree of uptake of drug across the vaginal mucosa.

1.1.6 Pathological Consideration

The vaginal epithelium becomes either thicker (hyperplastic) or thinner or ulcerated than normal during diseases states of vagina. They may affect the drug transport and bioadhesion. For example, at a time of menopausal diseases conditions, the vaginal wall tends to become thinner and the pH may also shift slightly. They may influence the drug permeability through the vaginal epithelium. In case of urogenital atrophy (Johnson J.R, 1989), the genital tract undergoes anatomical and physiological changes involves shrinkage of vagina and loss of rugae folds of the vagina that may reduce drug absorption by decreasing surface area. The cellular glycogen diminishes and Lactobacilli numbers decrease leading to a decreased lactic acid production and an increased vaginal pH. They can affect the penetration of ionizable drug through vaginal epithelium. Therefore, it is important to understand the nature of anatomical and physiological changes under relevant disease conditions before designing effective vaginal delivery systems.

1.1.7 Therapeutic Uses of Vaginal Drug Delivery

Most commonly use of vaginal drug delivery is in local treatment such as bacterial vaginosis (BV), vaginal candidiasis and other genital infections. Chances of adverse effects are very less in case of local application of certain antifungal/antibacterial drugs because of lack of systemic absorption.

Indeed, the first report of vaginal drug absorption was in 1918 (Barnhart K.T. *et al.*, 1918). Although the literature indicates Progesterins, Morphine, Pilocarpine, Bromocriptine (Varmesh M, 1988), Misoprostol (Zieman M. *et al.*, 1997), Prostaglandins (Gordon-Wright A.P. *et al.*, 1979), Naproxane (Constantine G. *et al.*, 1987), Metronidazole (Alper, M.M. *et al.*, 1985), d-Methazone (Swanson B.N *et al.*, 1978), Povidone-Iodine (Vorherr H. *et al.*, 1980), and Propranolol (Patel L.G, 1984) have been shown to be absorbed through the vaginal mucosa.

Despite the possible hepatic and gastrointestinal degradation, vaginal delivery offers a panorama of opportunities for delivering proteins/peptides and other active compounds that encounter degradation after oral administration. Various peptides, including Human growth hormone, Calcitonin (Richardson J. *et al.*, 1996), Oxytocin (Benziger D.P. *et al.*, 1983), Insulin (Richardson J. *et al.*, 1992b), and Steroids (Ralph L, 2001) have been delivered via vaginal route.

From anatomical and histological point of view, the vaginal route is well-suited site for delivery of microbicides, which can provide women controlled protective methodology against HIV and other sexual transmitted infections (STDs). The vaginal cavity is also an effective site for the uterine targeting delivery of various therapeutic agents such as terbutalin, progesterone and danazol, suggesting a new therapeutic modality in women's health care (Richardson J. *et al.*, 1992b)

Recently, the vagina has been studied as a novel route of delivery of chemotherapeutic agents for treatment of all cancers via transmucosal delivery system, which eliminates the need for parenteral administration (Pauletti G.M. *et al.*, 2002).

1.1.8 Pharmaceutical Aspects

1.1.8.1 Physiochemical Factors Associated with the Drug

Physiochemical parameters such as solubility, ionization characteristics, chemical structure, particle size and shape, stability of drugs, organoleptic properties of drug are important considerations during design and development of vaginal formulations (Fiese E., 1987). Physiochemical parameters governing formulation spreading, retention and release profile of drug. The release behavior of drug is depending on its solubility in simulated vaginal fluids,

which influence the onset and therapeutic efficacy of the drug. Water-soluble drug is a good candidate for vaginal drug delivery. If the drug is insoluble in aqueous fluids, its solubility can be increased by several mechanisms such as addition of solubilizing agents, cosolvency etc (Streng W. *et al.*, 1984). For example, a novel itraconazole formulation intended for vaginal use based on hydroxypropyl- β -cyclodextrin (HP β CD), a functional excipient that increases drug solubility (Peeters J. *et al.*, 2002). Ionization behavior of the drug within each compartment is governed by the pKa of the drug and the pH of that compartment in which it is present (formulation vehicle or site of administration). Acidic drug molecule maintains a high-unionized concentration at vaginal pH (ranges from 3.6 to 4.5). Therefore, it may serve as better candidate for the vaginal administration. Irritation potential of extreme acidic pH (less than 3.0) should be kept in mind at the time of design of acid buffering formulations (Kaminsky M., 1982). Existence of different physical states or change in form (amorphous or crystalline) of the drug may alter the biologic activity of the drug and formulation effectiveness during the processing and storage. Existence of physical states can be determined by techniques such as x- ray diffraction, optical crystallography, DSC etc (Byrn S. *et al.*, 1995). In some cases, particle size may even directly affect the efficacy of final formulation. Larger insoluble particles of active compound or excipients may also cause irritation to vaginal mucosa. Assessment of flow properties, dose uniformity, and dissolution rate are important aspects during the development of the vaginal formulations such as vaginal tablets, capsules, etc. These properties are largely affected by the particle size and shape of the drug and its excipients. The particle size of drug molecule can be determined by sieve analysis, laser diffraction technique, or coulter counter technique (Cartensen J., 1998). Molecular weight and size are important parameters that affect the drug release from dosage form, permeation of drug molecule through biological membranes into the systemic circulation. Lower molecular weight compound (less than 1000) is easily absorbed into systemic circulation via vaginal epithelium. For the local vaginal application, high molecular weight compounds (more than 1000) are preferred because of its lower permeability through vaginal mucosa. Molecular weight of drug may be determined by several methods (Billmeyer F. W., 1994) Hygroscopicity and moisture content of the active compound and excipients of the formulation considerably affects the dissolution behavior, stability of active compound, and the selection of dosage form as well as effective packaging system. The physical, chemical, and microbiological stability of the drug substances assumes as an important parameter of the formulation development due to it concern with shelf life of the formulation as well as toxicity associated with its degradation product. An organoleptic property of drug involves color, odor, and taste are important considerations for the formulation development

programme. In a survey conducted in Brazil, women of different age groups and socioeconomic status showed that most of women preferred a transparent or colorless vaginal formulation (Hardy E. *et al.*, 1998). Surface texture is also an important parameter for vaginal formulation due to avoidances of any damage to vaginal mucosa. Humectants, fine and uniform particle size of drug and excipients may be useful to make a smooth texture of formulations.

1.1.8.2 Factors Associated with the Dosage Form

Global research in pharmaceutical sciences will acquire new dimensions in the post- GATT (General agreement in trade and tariff) era. The many pharmaceutical industries currently focused on the development of novel vaginal drug delivery system for the prevention of undesirable conception, vaginal infections, sexual transmitted diseases (STDs) and cure of other gynaecological conditions. These innovative delivery systems also may enables to extend product life cycle and remains competitive in the market place. The alternative approach of such organizations would be to develop a formulations or new dosage forms using novel excipients for the existing drugs that offer distinct advantages over the conventional delivery systems.

Excipients in Vaginal Formulations: Selection of optimal excipients for vaginal formulations is a challenge to development scientists. Garg S. *et al.*, (2001) reported that excipients with different functionalities are used depending on the characteristics of the dosage form. Excipients can enhance the acceptable properties of formulation, which are required for masking the less desirable properties of the pharmacologically active constituents or improving acceptability of dosage form. Some additives can be incorporated to improve drug release pattern and absorption. Any component and/or excipients used in vaginal formulations needs to be approved for human use.

Drug Excipient Compatibility Studies: Any kind of incompatibilities between the drug-excipient and excipient- excipient not only affects the stability of the formulation, but also its performance to a significant extent. Hence, the drug-excipient compatibility studies are desirable for the selection of an optimal composition of the vaginal formulations. The compatibility between the drug and excipient can be easily evaluated by thermal and isothermal stress testing (Kandarapu R. *et al.*, 2001). Physical mixtures of drug with an individual excipient (in ratio present in final formulation) subjected to accelerated conditions and analyzed by differential scanning calorimeter (thermal technique) and isothermal method (HPLC).

Penetration Enhancer: Penetration enhancers are capable of promoting absorption and/or transport and penetration of the drug through the vaginal mucosa by decreasing penetration barrier of the vaginal mucosa. Currently, the most preferred penetration enhancers include non ionic surface active agents, bile salts, hyaluronic acid (Sandri et al., 2004) organic solvents, non-ionizable glycol ester derivatives such as polyethylene glycol, ethoxydiglycol known under its trade name TRANSCUTOL® and interesterified stone oil, also commercially available from Gattefosse. Penetration enhancers are used in vaginal formulation from about 2 to 30%, by weight. It is well known that bile salts play important role as physiological surfactants in absorption of lipids and lipid soluble vitamins. Bile salts have been extensively used to enhance the absorption of drugs through various mucosal membrane including vaginal epithelial. These compounds are acts by extraction of membrane protein or lipids membrane fluidization, and reverse micellization in the membrane.

Solubility Modifiers: The poor solubility of drug in simulated vaginal fluid may affect the release pattern of drug from its device, which influence the onset and therapeutic efficacy of the drug. The most commonly used solubilizing agent for intravaginal formulations include complex-forming solubilizer such as citric acid, ethylenediamine-tetraacetate, polyvinylpyrrolidone, micelle-forming solubilizers such as tweens and spans (for example tween 80), polyoxyethylene, sorbitan, fatty acid ester, polyoxyethylene n-alkyl ethers, poloxamers, organic solvents, phospholipids and cyclodextrin (Pauletti et al., 2002).

1.1.9 Carriers for Drug Delivery

Use of appropriate delivery system is very important for the vaginal drug administration. Delivery system should be able to maintain the desired therapeutic amount of drug at the site of action for a specified time period. Retention time and stability of drug in vaginal cavity is the most challenging task for any delivery system.

Tablet and Suppositories- Tablet and Suppositories are most commonly available carrier for vaginal drug delivery. These formulations are designed to melt in the vaginal cavity and release the drug into vaginal cavity. Suppositories are most commonly used to administer drugs for cervical ripening prior to child birth and for local delivery of various drugs. Mucoadhesive polymers are sometimes used in tablet formulations to increase the vaginal residence time of the drug been delivered.

Creams and Gels- To date the greatest number of carrier in the form of creams or gels are used for intravaginal delivery of drugs. Traditional medicines are being formulated as vaginal formulations. For example, V-gel (Himalaya Drugs Company, India), which is an ayurvedic vaginal formulation for the treatment of candidiasis, trichomoniasis, bacterial vaginitis.

Gels and creams are being investigated more extensively (Kieweg *et al.*, 2004). For example, glyminox gel for contraception and prevention of sexually transmitted infections (STIs), terbutaline gel for dysmenorrhea and endometriosis. To evaluate the efficacy of a 3-day course of clindamycin vaginal cream in the treatment of bacterial vaginosis, Lamont *et al.*, (2003) performed a randomized, placebo controlled trial in pregnant women and found optimum dose that will necessary to ensure protection. Although commonly used for the topical intravaginal delivery of drugs, these systems are messy, uncomfortable and may not provide an exact dose due to non-uniform distribution and leakage (DuBouchet *et al.*, 1998). Advances have been made by using mucoadhesive polymers to minimize leakage and improve retention to the vaginal epithelial. Francois *et al.*, (2003) reported a mucoadhesive, cyclodextrin-based vaginal cream formulation of Itraconazole, was found to form a thin bioadhesive layer over the genital tract surface in phase III clinical trials. Clinical studies found that the formulation was well tolerated and effective in combating vaginal candidiasis. Robinson *et al.*, 1994 reported the mucoadhesive gels based on polycarbophil (Repelen gel) was to be retained in the genital cavity for 3-4 days.

Microemulsion- Microemulsion-based formulations that offer rapid dispersion and enhanced drug absorption profile can be exploited for the development of novel vaginal delivery system (Eccleston G.M *et al.*, 1992). Microemulsions are thermodynamically stable, isotropically clear dispersions of water, oil, and surfactants with potential as drug-delivery vehicles. Microemulsions appear to have the ability to deliver larger amounts of topically applied agents into the mucosa than do traditional lotions and creams because they provide a better reservoir for a poorly soluble drug through their capacity for enhanced solubilization. For example, D'Cruz *et al.* (2001) developed GM-144 a novel lipophilic gel-microemulsion as a vaginal contraceptive by using commonly used pharmaceutical excipients through systemic mapping of ternary-phase diagrams

Microsphere- Microspheres are good choice for vaginal delivery because of its protective shield and sustain release property. Mucoadhesive polymers can be used in the preparation of microsphere to prolong the residence time. HYAAF microspheres have great potential for systemic delivery. Benziger *et al.*, (1983) reported that salmon calcitonin incorporated in HYAAF microspheres was observed to facilitate the rate of vaginal absorption as compared to aqueous solution of the drugs.

Thermoreversible gel – The timely gelation and retention of in situ-gelling vaginal formulations would be fundamental to improve the efficacy of drugs. Roy *et al.*, (2001) reported that phase changes polymers composed of polyoxypropylene and polyoxyethylene are used to form thermoreversible gels when incorporated into aqueous solutions. These

polymers exhibit sol-gel transition in response to body temperature, pH and specific ions, therefore allowing advantageous topical applications. The liquid state-to-gel phase transition is dependent on the polymer concentration and the ingredients incorporated into the solution. Chang, J.Y. *et al.*, (2002) have recently reported a mucoadhesive thermosensitive gel (combination of poloxamers and polycarbophil) that exhibited increased and prolonged antifungal activity of clotrimazole in comparison with conventional PEG-based formulation.

1.1.10 Experimental Models

Ex vivo model – Many type of cell lines and excised tissues (excised from pig, monkey, cow, and bovine, sheep tissue) are used for evaluation of bioadhesion and cellular viability of vaginal formulation. Most common cell lines used are HeLa-S3 cell lines. Ex Vivo experiments include the determination of drug retention and bioadhesive characteristics in addition to various physical and chemical properties of formulations. The retention behavior of vaginal formulation can be determined by using simulated dynamic vaginal system. Simulated dynamic vaginal system (Jin-Wook Yoo *et al.*, 2006) consisted of closed glass cell with 30° angle slope and flow rate pump as shown in Fig. 1.2.

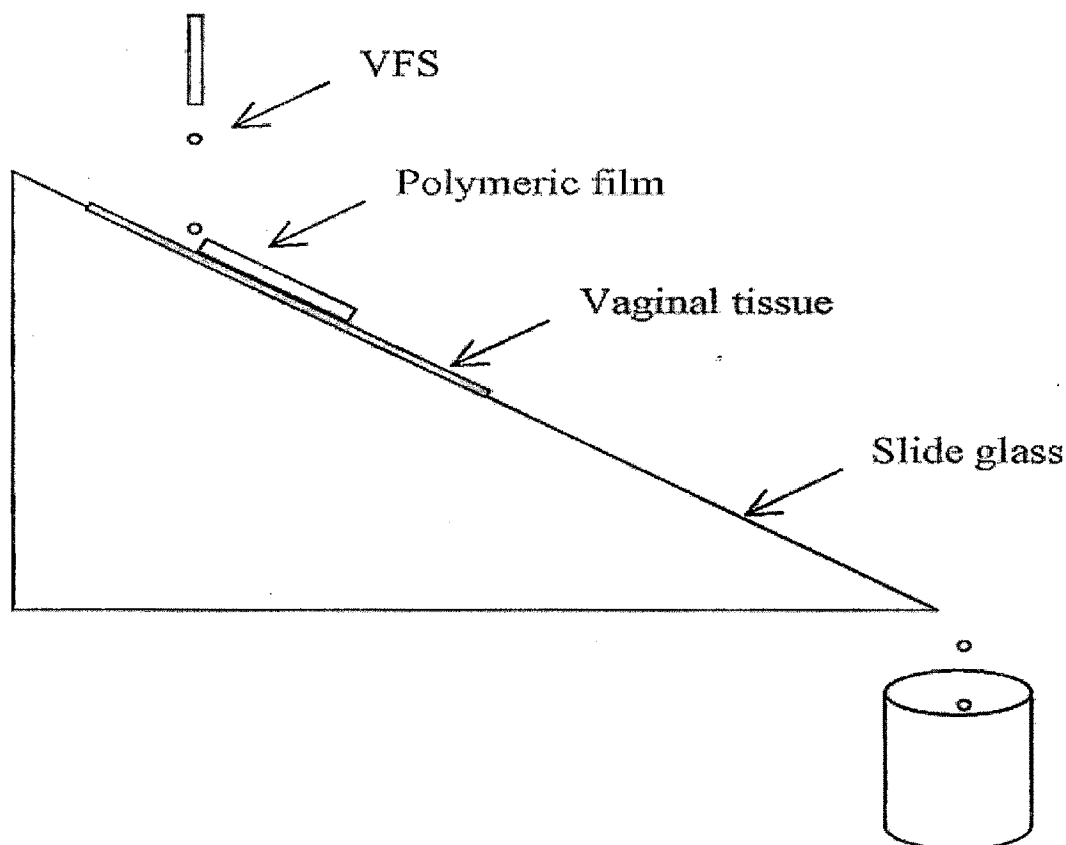


Fig. 1.2 Simulated dynamic vaginal system simulated vaginal

The bioadhesive strength of the vaginal formulation can be measured by texture analyzer as shown in Fig.1.3.

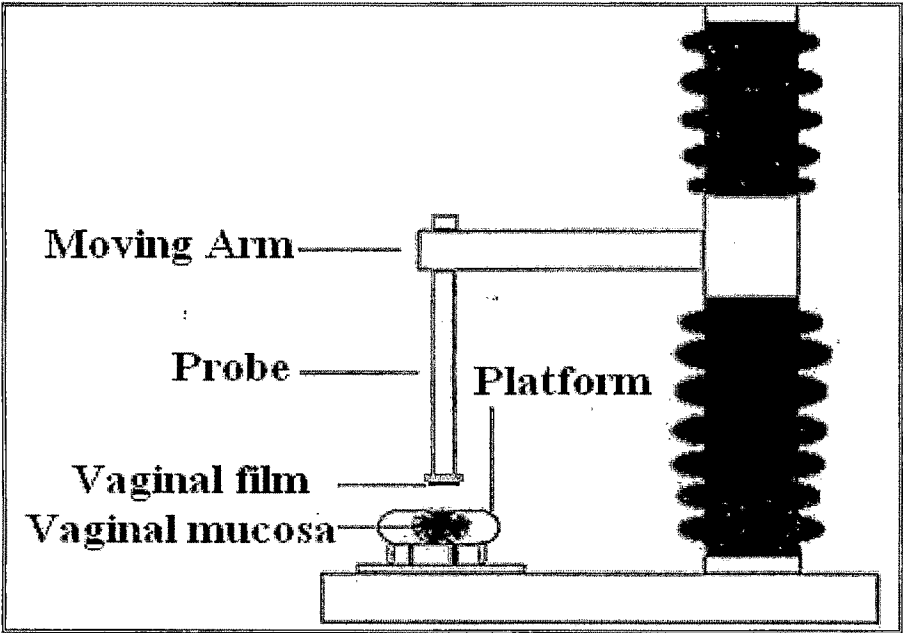


Fig. 1.3 Outline diagram of texture analyzer showing the set up of in vitro bioadhesion experiment.

In vivo model- In vivo model are very expensive to do. Also results vary from species to species. Several animal models such as sheep, rat, rabbit, rhesus monkey, macaque monkey, dog and mice have been used in different studies. The selection of animal model is dependent upon the type of study. White rabbits are used for primary irritation and subchronic toxicity testing of vaginal formulation. Generally small animals are very commonly used due to less cost.

In vivo studies are carried out for the assessment of efficacy, distribution, spreading and retention of formulations in the vagina (Crabb C., 2003). Gamma scintigraphy and colposcopy (Patton D.L. *et al.*, 1998) are desirable techniques for assessing the distribution, spreading and retention of vaginal formulations in sheep and human. However, the significance of these findings is still under debate. Two imaging techniques are being developed to measure the degree of coverage in the vaginal vault: magnetic resonance imaging (MRI) and an optic probe inserted vaginally (Barnhart K.T. *et al.*, 2006).

Table 1. List of Vaginal Preparations Recently Developed or Under Development

Product name/Dosage Form	Active Ingredient	Manufacturer or Developed by	Status
Prochieve™ 4% (Bio-adhesive gel)	Progesterone	Columbia laboratory	Market (USA)

Product name/Dosage Form	Active Ingredient	Manufacturer or Developed by	Status
Gyno-V (Softgel capsule)	Neomycin Sulfate Polymoxin B Sulphate Nystatin	Unimed Pharm, Inc.	Market
Camazole (Tablet)	Clotrimazole	Keun Wha Pharmaceutical Ltd.	Phase II/III clinical trial
Emmelle (gel)	Dextrin sulphate	M - L Laboratory	Phase III clinical trial
Cervidil (Insert)	Dinoprostone	Forest Pharmaceutical Inc.	Market
Savvy TM (gel)	Glyminox	Biosyn	Phase III clinical trial
Vaginal contraceptive Film	Nonxynol-9	Apothecus Pharmaceutical	Market
Estring (Vaginal ring)	Estrogen	Pharmacia & Upjohn Company	Approved (1998)
Gynol II (Jelly)	Nanoxynol -9	Ortho -McNeil	Market
KY Plus (Jelly)		Johnson & Johnson	Market
Miconazole nitrate Ovule	Miconazole nitrate	Personal Care Products, NJ	Phase IV
Protectaid (Sponge)	Nanoxynol- 9 Benzalkonium chloride	Axcan pharma	Market
Invisible condom	Sodium lauryl sulphate	Laval university	Phase I/II clinical trial
Viread (gel)	Tenofovir	Gilead Science	Phase II clinical trial
Ushercell (gel)	Cellulose sulphate	Polydex Pharmaceuticals	Phase III clinical trial
Carraguard (gel)	Carrageenan/ PC-515	Population Council	Phase III clinical trial
Placebo gel	Hydroxy ethyl cellulose	Biosyn	Phase I clinical trial
Vaginal gel	Cellulose sulphate	Family healthcare	Phase III clinical trial
Pro 2000 (gel)	Naphthalene2-sulfonate	Indevus Pharmaceuticals, Inc.	Phase III
Vagifem® (tablet)	Estrogen	Novo Nordisk	Market
Crinone (gel)	Progesterone	Wyeth-Ayerst laboratories	Approved (1997)
Gynazole-1(cream)	Butoconazole nitrate 2%	KV Pharmaceuticals, USA.	Market
Clindesse vaginal cream	Clindamycin phosphate	KV Pharmaceuticals, USA.	Market

Product name/Dosage Form	Active Ingredient	Manufacturer or Developed by	Status
Prochieve TM 4% (Bio-adhesive gel)	Progesterone	Columbia laboratory	Market (USA)
Advantage- S (gel)	Nonxynol-9	Columbia laboratory	Market (USA)
Chronodyne TM (Bio-adhesive gel)	Terbutaline	Ardana bioscience Ltd	Phase II clinical trial
Lidocaine (gel)	Lidocaine	Columbia laboratory	Phase III clinical trial
Testosterone (gel)	Testosterone	Columbia laboratory	Phase III clinical trial
Prepidil (cervical gel)	Dinoprostone	Pharmacia & Upjohn Company	Market
Culturelle VC (capsule)	Probiotics	Pharma Dyanemics (Pvt.) Ltd.	Market

HPMC= Hydroxy propyl methyl cellulose, MHPC = Methyl hydroxy propyl cellulose,
PEG = polyethylene glycol

1.1.11 Challenges and Future Prospects

Vaginal drug delivery system offers a lot of advantages for local and systemic treatment. The conventional use of vaginal drug delivery in local treatment such as vaginal infections is well established. Most of the available vaginal delivery systems are associated with problems like messiness, leakage and poor retention at site of administration causing inconvenience to users, leading to poor patient compliance. Novel vaginal delivery system designed with bioadhesive polymers can overcome some of the key limitations associated with conventional delivery of vaginal drugs. A direct relationship has been found between the amount of bioadhesive polymers used and local toxicity. Therefore, bioadhesive polymers should be thoroughly tested for toxicity before its use in any vaginal formulation. Extensive research is needed to find out the chronic toxicity produced by these polymers because so far most of the toxicity study has been limited to its acute effects.

Future of vaginal drug delivery seems to be very bright. From past few decades many of the vaginal products have come in the market and few of them are under clinical trials. Continuous studies are being carried out for the development of peptide and vaccine delivery. Also, vaginal drug delivery is a promising area for continued research with the aim of delivery of microbicides that can prevent transmission of sexually transmitted diseases (STDs)/ HIV.

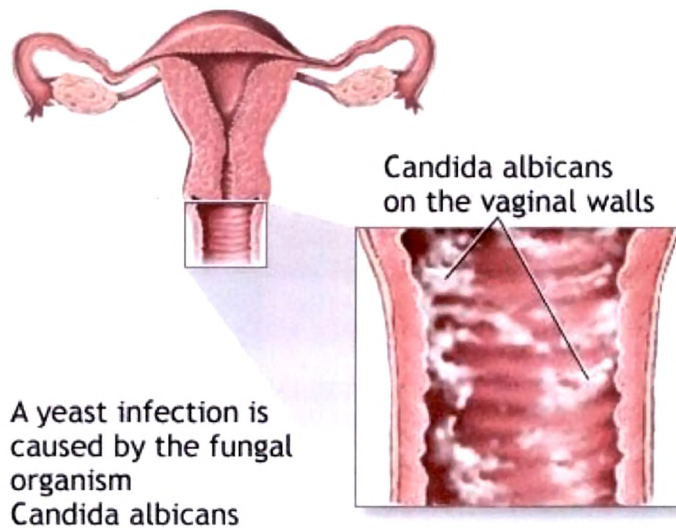
Table 2. Summery of recent advances in the vaginal drug delivery

Drug	Formulation	Result/Purpose of investigation	Reference
Danazol	T- shaped preperation IUD- ring preperation Paste/ Spherical gel	Novel pharmaceutical preparation for the treatment of gynecological diseases	Ikuo S. <i>et al.</i> 2006
Insulin	Microsphere	Increased absorption from HYAFF microsphere as compared to aqueous solution of the drugs	Lllum L. <i>et al.</i> 1994
Salmon calcitonin	Microsphere	Increased absorption from HYAFF microsphere as compared to aqueous solution of the drugs	Richardson J.L. <i>et al.</i> 1996
Acriflavine	Microsphere	Controlled Release	Gavini E. <i>et al.</i> 2002
Leuporelin Acetate	Intrauterine Device (IUD)	Prolonged release of Leuporelin acetate	Nonomura M. <i>et al.</i> 1998
Metronidazole	Tablet (Bioadhesive)	Mucoadhesive dosage form	Bouckaert S., 1995
Benzylamine Hydrochloride	Vaginal gel	Provide a release of the active agent over an extended period of time about 12 to 36hrs	Borgman R.J. <i>et al.</i> 2005
Cellulose acetate Sulphate	Sponge disc	Provide a biodegradable microbicidal vaginal barrier device for the prevention of STDs	Neurath A.R. <i>et al.</i> 2003
Clotrimazole	Liposome gels (Bioadhesive)	Novel delivery system for local therapy of vaginal infection	Ning M. <i>et al.</i> 2005
Clotrimazole	Tablet (Bioadhesive)	The water uptake and cohesive properties of vaginal tablets consisting of these new conjugates could be significantly improved.	Kast C.E. <i>et al.</i> 2002
Chloramphenicol	Liposome gels (Bioadhesive)	Enabled as sustained release of drug and novel delivery system for local treatment of bacterial vaginosis.	Eljka P. <i>et al.</i> 2004
Clotrimazole	Pessaries	Good adhesion properties and the capacity of hold the dosage form in the target site.	Ceschel G.C. <i>et al.</i> 2001
Oxybutynin	Vaginal ring	Controlable release of drug upto several days on single application	Woolfson A.D. (2003)
Acyclovir	Liposomal hydrogel (Bioadhesive)	Provide sustained release and improved bioavailability	Pavelie Z. <i>et al.</i> 2005
Progesterone	Vaginal Ring	Advanced delivery system of hormone replacement for a human female	Pekka L. <i>et al.</i> 2000
Ethinyl estradiol Etonogestrel	Vaginal ring	Reliable release of two or more active substances in a substantially constant ratio to one another over a prolonged period of time can be achieved using a one-compartment	Roumen F.J.M.E. <i>et al.</i> 2001
Physiological active Peptides	Gel, Tablet, Film Soft capsule	Excellent absorbability of the active ingredient	Inamoto S. <i>et al.</i> 1998

1.2 BACTERIAL VAGINITIS

1.2.1 Vulvovaginal Candidiasis

Vaginal candidiasis is a fungal or yeast infection of the vulva and/or vagina. It causes a smelly, thick, white-yellow discharge that might be accompanied by itching, burning and swelling. It can also make walking, urinating and/or sex very painful. Vaginal candidiasis can be an occasional problem for even the healthiest woman. However, it's more common and severe in women with weakened immune systems. For many, a repeating or worsening vaginal yeast infection is the first symptom of HIV infection. This infection can occur at any CD4+ cell count but is likely to occur more often when your CD4+ count falls below 100.



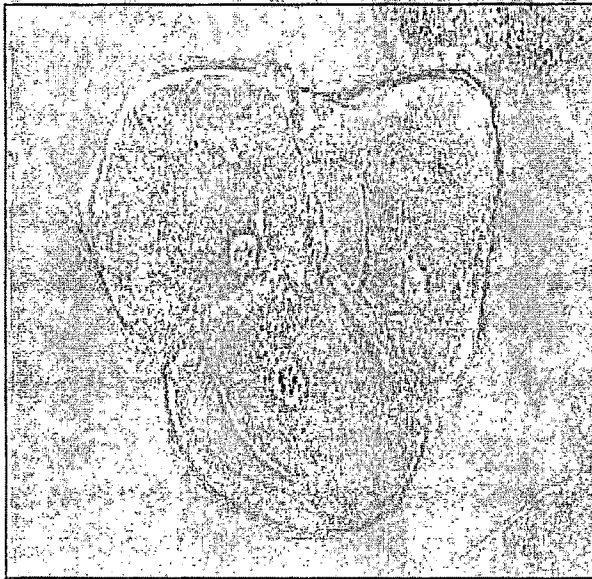
Cause

Vaginal candidiasis is caused by *Candida species*. Everyone has small quantities of the fungus in the mouth, vagina, digestive tract and skin. In healthy persons, "friendly" bacteria and the immune system prevent the fungus from causing infection. However, if you have a damaged or weakened immune system, it's easier for *Candida* to grow and cause disease. Certain drugs can alter the natural organisms in the vagina, which can then promote the growth of *Candida*. These include the extended use of antibiotics, steroids and oral contraceptives (birth control) with high estrogen content. Other factors that may cause candidiasis include: diabetes, pregnancy, using antihistamines (drugs commonly used to prevent allergies and rash) and iron, folate, vitamin B12 or zinc deficiency. Factors that may weaken the immune system from cancer chemotherapy to stress and depression can also cause candidiasis. Tight fitting pants and reactions to chemical ingredients found in soaps and detergents can lead to vaginal candidiasis as well.

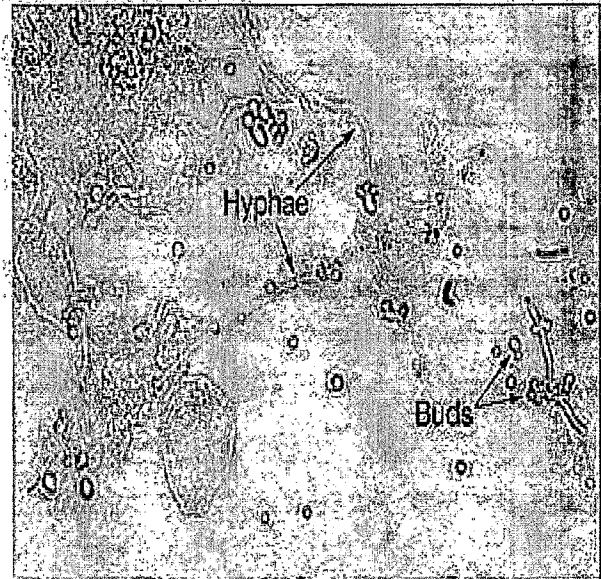
Diagnosis

Vaginal candidiasis is usually diagnosed by appearance and symptoms. Because symptoms are similar to many other conditions, like the sexually transmitted disease trichomonas, your doctor should confirm a diagnosis by scraping an affected area for examination under a microscope. Other lab tests are usually done if the infection does not clear up after treatment.

A Normal Vaginal Epithelial Cells



B Candida



Treatment

Topical treatments (active only on the area where applied) are the first choices for yeast infections and these generally work for mild-to-moderate cases. These include vaginal creams, suppositories or tablets. Many are available over-the-counter in a drugstore.

Most topical treatments are put into the vagina once or twice a day for three days or once a day for seven days. (See Table 3. for drug names and doses). Longer courses (7-14 days) may be more effective in HIV-positive women. Generally, topical treatments do not cause side effects, but in a small number of women they may lead to vaginal burning, itching or skin rash. A few women have experienced cramps or headaches. **Oil-based vaginal creams should be used with caution as they may weaken latex condoms and diaphragms.**

Anti-Fungal Drugs and Pregnancy

The U.S. public health service *Guidelines for the prevention of opportunistic infections* recommend that oral azole anti-fungals including fluconazole (Diflucan), itraconazole (Sporanox) and ketoconazole (Nizoral) should not be used during pregnancy because they have caused birth defects in animal studies. In case of treatment of vaginal candidiasis in pregnant women, topical therapies are more preferable because of less systemic side effect.

Table 3. Treating Vaginal Candidiasis

Drug Name	Dose	Notes
Topical Therapies		
Butoconazole 2% cream	5 grams for 3 days	Available over-the-counter. May weaken latex condoms and diaphragms.
Clotrimazole (Lotrimin) 1% cream	5 grams for 7-14 days	Available over-the-counter. May weaken latex condoms and diaphragms.
Clotrimazole (Mycelex) 500mg vaginal tablet	Single dose	
Miconazole (Monistat) 2% cream	5 grams for 7 days	Available over-the-counter. May weaken latex condoms and diaphragms.
Miconazole (Monistat) 200mg vaginal suppository	Once a day for 3 days	Available over-the-counter.
Miconazole (Monistat) 100mg vaginal suppository	Once a day for 7 days	Available over-the-counter.
Tioconazole (Vagistat) 300mg ointment	A single dose	Available over-the-counter.
Terconazole (Terazol 7) 0.4% cream	5 grams for 7 days	May weaken latex condoms and diaphragms.
Terconazole (Terazol 3) 0.8% cream	5 grams for 3 days	May weaken latex condoms and diaphragms.
Oral Therapies		
Fluconazole (Diflucan)	One 150mg dose	Not recommended for pregnant women; sometimes used weekly as a preventive measure.
Itraconazole (Sporanox)	One 100mg dose	Not recommended for pregnant women; sometimes used weekly as a preventive measure.
Ketoconazole (Nizoral)	One 200-400mg dose	Not recommended for pregnant women; sometimes used weekly as a preventive measure.
Other Therapies		
Nystatin vaginal tablet (Mycostatin)	Applied to affected areas twice a day for three days.	Available over-the-counter. May be useful for recurrent infections (apply every 7 days for 1 month); messy application; can cause vaginal swelling; refraining from sex or use of condom recommended.

1.2.2 Bacterial Vaginosis

The vagina is a unique environment for bacterial colonization. It is subject to dramatic changes over the course of a lifetime, induced by developmental and hormonal changes. At birth, it is lined by stratified squamous epithelium, which regresses as the influence of maternal estrogen wanes. In childhood, the vaginal flora contains skin commensals and bowel organisms. At menarche, the pH falls from neutral to approximately 4, and the flora becomes dominated by lactobacilli. Many other organisms may be present in lower concentrations, including anaerobic and facultative anaerobic bacteria and *Candida* spp. The hormonal environment alters on a monthly basis, with additional disturbances to the ecosystem produced by menstruation, washing, and hygiene. Sexual activity can introduce a number of new species and pathogens, as well as alter the pH. Pregnancy and breast-feeding produce longer fluctuations in the hormonal balance.

BV can be thought of as a disturbance in this vaginal ecosystem in which the lactobacilli are replaced by a variety of anaerobic bacteria and mycoplasmas. It may be transient or become persistent. It is recognized as the most common cause of abnormal vaginal discharge in women of childbearing age. Common agents of BV include *Gardnerella vaginalis*, *Mobiluncus*, *Bacteroides* spp. and *Mycoplasma hominis*. The term 'BV' was agreed at an international symposium in 1983, replacing the older term 'Gardnerella vaginitis'. This recognized the fact that many anaerobic or facultative anaerobic bacteria are present and that classical signs of inflammation are absent. The diagnosis is usually confirmed using the composite (Amsel) criteria (see below). About 50% of cases appear to be asymptomatic. Studies in the last decade have established that BV is associated with infective complications in pregnancy and is a risk factor for the acquisition of HIV.

Epidemiology

In unselected populations, the prevalence of BV is 10–20%, but it may be as high as 36% in women attending STI clinics and 28% in those seeking elective termination of pregnancy. BV is probably more common in women with STIs and in those who have recently changed sex partner, but it has been reported in virgin women. In many studies, it is associated with IUD use. The condition often arises spontaneously around the time of menstruation, and may resolve spontaneously in mid-cycle. It is not known how often BV occurs in post-menopausal women.

Vaginal Physiology and BV

At menarche, under the influence of estrogen, stratified squamous epithelium develops in the vagina. Lactobacilli become the dominant organism. The source of the vaginal lactobacilli in an individual woman has not been determined. Lactic acid is produced by both bacterial metabolism and that of the epithelium, and the vaginal pH falls to a level usually between 4.0 and 4.5. Physiological discharge consists of mucus, desquamated epithelial cells, and lactobacilli. The pH may rise above 4.5 at the time of menstruation, when the concentration of lactobacilli is reduced. Cervical mucus and semen have pH between 7 and 8. In mice, exogenous treatment with progesterone alters the flora to one that resembles BV (Taylor-Robinson D *et al.*, 1997). How much do the hormonal changes of a menstrual cycle influence the flora in women?

If BV develops, the pH rises to a level between 4.5 and 7.0. The anaerobic or facultative anaerobic organisms which are usually present in low numbers increase by between 100- and 1,000-fold, to considerably outnumber the lactobacilli, which may eventually disappear. Trimethylamine and the polyamines putrescine and cadaverine are produced by anaerobic metabolism, and they are thought to be responsible for the fishy smell. Microscopy of vaginal fluid shows multiple small bacteria and epithelial cells with large numbers of adherent bacteria. Gardner and Dukes called these clue cells, as they gave a clue to the diagnosis of nonspecific vaginitis (Gardner H. L. *et al.*, 1955).

Diagnosis

BV should be suspected in any woman presenting with an offensive, typically fishy-smelling vaginal discharge. Speculum examination shows a thin, homogeneous, white or yellow discharge adherent to the walls of the vagina. Gardnerella can be found in low concentrations in more than 50% of women without BV; therefore, culture has a poor specificity and should not be used for routine diagnosis.

Amsel criteria for the diagnosis of BV

- Vaginal pH > 4.5
- Release of a fishy smell on addition of alkali (10% potassium hydroxide)
- Characteristic discharge on examination
- Presence of 'clue cells' on microscopy

At least three of the four criteria must be fulfilled to make a diagnosis of BV. In routine practice, vaginal pH can be measured using pH-sensitive paper. A pH of less than 4.5 almost excludes BV. If the pH is high, send a high vaginal swab to the microbiology laboratory. This

should be examined by wet mount or Gram-staining. **Gram-staining** examination of a Gram-stained vaginal smear is a quick and relatively simple means of confirming the diagnosis of BV. Typical lactobacilli are large, Gram-positive rods with blunt ends. Gardnerella is usually a Gram-negative coccus. The normal flora includes plentiful lactobacilli, whereas in BV there are large numbers of Gram-negative cocci and small rods. Scoring systems (Nugent *et al.*, 1991) have attempted to reduce inter-observer variability. Recently, a simplified scoring system (Hay-Ison criteria) has been recommended for use in GUM clinics in preference to the Amsel criteria (Ison C. A. *et al.*, 2002).

Differential diagnosis of vaginal discharge

Symptoms and signs	Candidiasis	BV	Trichomoniasis	Cervicitis
Itching or soreness	++	–	+++	–
Smell	May be 'yeasty'	Offensive, fishy	May be offensive	–
Colour	White	White or yellow	Yellow or green	Clear or coloured
Consistency	Curdy	Thin, homogeneous	Thin, homogeneous	Mucoid
Other signs	Purulent mucus at cervical os			
Potassium hydroxide test	–	++	±	–
pH	< 4.5	4.5–7.0	4.5–7.0	< 4.5
Confirmation	Microscopy and culture	Microscopy	Microscopy and culture	Microscopy, tests for Chlamydia and gonorrhoea

Microbiology

The organisms most commonly associated with BV are *G. vaginalis*, *Bacteroides* (Prevotella) spp., *Mobiluncus* spp., and *Mycoplasma hominis*. High concentrations of Gardnerella, >100-fold greater than normal, are found in up to 95% of women with BV, but Gardnerella was also found in more than 50% of women without BV, so culture has a poor specificity. Quantitative culture showing high concentrations correlates better with BV in research studies, but culture should not be used for routine diagnosis. The reported prevalence of other organisms often reflects the sensitivity of the culture method for the specific organism. For instance, *Fusobacter* spp., peptostreptococci, and non-*viridans* group streptococci have also been associated with BV.

One study looked at the changes in bacterial flora that occur as it passes from normal to intermediate and BV (Rosenstein I.J. *et al.*, 1996). At the intermediate stage, *Gardnerella* and *Bacteroides* were present in moderate concentrations, but high concentrations of those

organisms and of *M. hominis* were not seen until full BV had developed. The presence of high concentrations of *Mobiluncus*, visible on a Gram stain, has been used to define the most abnormal flora, with a Nugent score of 9 or 10. Recently, by use of PCR, *Mobiluncus* spp. was detected in 84.5% of women with BV and 38% of those without BV (Schwebke J.R. *et al.*, 2001).

Lactobacilli

Lactobacilli produce a variety of substances, such as bacteriocins and lactocins, which are toxic to other bacterial species and lactobacilli, respectively. Acidification of the vagina is an important defense mechanism. H_2O_2 is also an important inhibitor of anaerobic growth, and it is thought that *Lactobacillus* spp. producing high levels of H_2O_2 provide protection against BV and acquisition of sexually transmitted infections. Thus, in vitro at a pH of <4.5, H_2O_2 -producing lactobacilli inhibit the growth of BV-associated organisms effectively, but at higher pHs the effect wanes (Klebanoff S. J. *et al.*, 1991).

Is a change in pH such as occurs at the time of menstruation and following unprotected intercourse sufficient to trigger BV? Alternatively, if sufficient numbers of bacteria are inoculated into the vagina from a partner, will BV develop? Another possible trigger is anything that reduces the number or quality of lactobacilli. Interestingly, broad-spectrum antibiotics that inhibit lactobacilli do not seem to trigger BV (Agnew K. J. *et al.*, 1995), possibly because they also inhibit some of the BV organisms.

An absence of healthy H_2O_2 -producing lactobacilli might contribute to frequent recurrences of BV in some women. One approach under study is to recolonize the vagina with healthy lactobacilli. In a small study from Belgium, 32 women with BV or intermediate flora were treated with vaginal tablets containing 50 mg of a lyophilisate of viable, H_2O_2 -producing *L. acidophilus* and 0.03 mg of estriol for 6 days (Parent D. *et al.*, 1996). There was a significant benefit, with a cure rate after 6 weeks of 88% in the treatment group compared to 22% in the placebo group.

Management

BV is sometimes distressing and must be managed with sensitivity. Because it has a relapsing–remitting course in many women, the value of treating asymptomatic BV has not been established. There is also no evidence that treatment reduces the prevalence in the

community. Treatment should therefore be prescribed for control of symptoms, and in situations in which it might prevent complications pregnancy.

Antibiotics with good anti-anaerobic activity should be an effective treatment for BV, and metronidazole and clindamycin are obvious choices. However, clindamycin has better activity against *G. vaginalis*, *Mobiluncus* spp. and *M. hominis* than does metronidazole. The standard treatment for BV is metronidazole, 400 mg b.d. for 5 days. An alternative is a 2 g single dose. The cure rate immediately after treatment with metronidazole is up to 95%, but after 4 weeks this declines to 80% in open-label studies and less than 70% in blinded studies. Topical treatments with intravaginal 2% clindamycin cream or 0.75% metronidazole gels are licensed for the treatment of BV.

Adverse effects of treatment – Oral metronidazole is associated with well-recognized side-effects of nausea, a metallic taste and alcohol intolerance. Allergic rashes occur occasionally. Oral clindamycin can induce rashes and occasionally pseudomembranous colitis. About 10% of women develop symptomatic candidiasis following treatment of BV.

Complications

Pregnancy: BV is associated with second-trimester miscarriage and preterm birth. Several studies have evaluated the value of screening for and treatment of BV in preventing adverse outcomes in pregnancy. The results have been variable; some studies show a benefit with treatment in terms of reducing preterm birth rates, but the largest study to date showed no benefit from treatment with short courses of metronidazole.³ On the basis of these studies, it cannot be concluded that antibiotic treatment of BV in pregnancy will universally reduce the incidence of preterm birth. This was confirmed by the most recent meta-analysis (Okun *et al.*, 2005)

Termination of pregnancy: Women infected with *Chlamydia trachomatis* who undergo elective termination of pregnancy are at high risk of endometritis and pelvic inflammatory disease. BV also confers an increased risk, and may be present in almost 30% of such women. A double-blind, placebo-controlled trial in Sweden showed that the risk of endometritis in women without *Chlamydia* was 12.2% in a placebo-treated group and 3.8% in those prescribed oral metronidazole before termination (Thejls H. *et al.*, 1992). A more recent randomized controlled trial in Sweden found a fourfold reduction in infective complications with clindamycin cream compared with placebo (Dalaker K. *et al.*, 2002).

Other gynaecological surgery: BV has been associated with vaginal cuff cellulitis, wound infection and abscess formation after hysterectomy. No randomized trials have been performed to investigate the value of screening and treatment before such surgery. The

potential role of BV in infections following IUD insertion, hysteroscopy, and dilatation and curettage has not been systematically studied.

HIV and STIs: HIV has spread rapidly through Sub-Saharan Africa and South East Asia in the last two decades. Initial reports identified genital ulcer STIs as co-factors for transmission. BV emerged as a co-factor for HIV acquisition in the Rakai study in rural Uganda (Sewankambo N. *et al.*, 1997). A study of pregnant women in Malawi reported BV to be associated with HIV acquisition during pregnancy and the postnatal period (Taha T.E. *et al.*, 1998). Potential mechanisms by which BV might increase HIV transmission include effects on local immune mediators. Additionally, hydrogen peroxide produced by lactobacilli can inhibit HIV in vitro, and is absent in most women with BV. If BV is established as an important risk factor for HIV spread, its control will become an important public health issue in many countries. BV has also been associated with an increased incidence of non-gonococcal urethritis in male partners.

Prevention

Because the aetiology of BV is not fully understood, it is not known how to prevent it. Antibiotics inhibit growth of the anaerobes, but do not necessarily eliminate the factors that led to the development of BV; therefore, relapse is relatively common.

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