Introduction and Review of Literature

Sexually transmitted infections (STIs) and unintended pregnancies are the leading causes of morbidity and mortality among women of child bearing age (Tsui et al., 2010, Ebrahim et al., 2005). An estimated 448.3 million cases of curable STIs occur annually worldwide, and with 248.5 million cases trichomoniasis has the highest incidence of 55.2% (WHO, 2011). On the other hand, of 208 million pregnancies that occurred globally in 2008, 41% were unintended (Singh et al., 2010). It has been seen that the primary responsibility of pregnancy and sexually transmitted disease (STD) protection lies with the female partner during most of the heterosexual contacts, including the “most vulnerable” contacts among adolescents and promiscuous adults (Chopra et al., 2007). Often under such circumstances condom (the only available dually protective contraceptive) is either inaccessible or its use is practically not negotiable. Consequently, woman-controlled, dually protective contraceptives that are safe, effective, and virtually imperceptible are highly desirable to curb the unintended pregnancy and STD/HIV ‘epidemics’.

The decline in oral contraceptive and diaphragm use and an increase in reliance on condoms and spermicides during recent times suggest that concerns about HIV and other STDs are changing patterns of contraceptive usage among sexually active adolescent and adult women worldwide (D’Cruz and Uckun, 1999). This indicates that in future women would depend heavily on spermicidal microbicides for ultimate safety during heterosexual contacts. Presently, all over-the-counter vaginal contraceptives incorporate a strong detergent (mostly nonoxynol-9) as the active ingredient for killing sperm by a general, non-specific cytotoxic surfactant action. However, the recent revelation about surfactants increasing susceptibility to HIV and STDs by their detergent action on vaginal and cervical epithelium has made them unsuitable for vaginal use. Nonoxynol-9 (N-9), the strong non-ionic surfactant spermicide found in most of the vaginal contraceptives and some sexual lubricants, has been shown to cause vaginal/cervical irritation, inflammation and ulceration, resulting in increased...
susceptibility of users to HIV infection (Stephenson 2000, Van Damme et al., 2002). The WHO has cautioned against the use of N-9 as a microbicidal spermicide by individuals who are at a high risk of acquiring HIV. Therefore, an unanticipated void has been created as no other safe spermicide is available to replace N-9 (and other detergents) in vaginal contraceptive preparations.

In the midst of global epidemics of both unwanted pregnancies and sexually transmitted diseases (STDs, including HIV) the options are very limited for the women. STD and HIV infections spread more rapidly from men to women than from women to men (D’Cruz and Uckun, 2001). On the other hand, human population is increasing steadily and the current global population of ~7 billion and it is increasing at the rate of 80 million persons a year. World population is projected to increase to ~9 billion by the year 2050 (Godfray et al., 2010). Serious research is thus warranted to empower women against these reproductive ‘threats’ in the twenty first century.

**World Population Statistics**

World population is estimated to number 7.026 billion by the United States Census Bureau (USCB, 2012). The USCB estimates that the world population exceeded 7 billion on March 12, 2012. The population explosion has been a major global concern that poses significant threat to the quality of life, more particularly in the underdeveloped and developing countries. Despite the availability of a number of contraceptive options, globally 100 million unwanted pregnancies occur every year. Approximately 400,000 teens aged 15-19 years give birth every year in the United States (Hamilton et al., 2010), and the teen birth rate remains the highest in the developed world (UN, 2010). Unintended pregnancy remains an alarming global public health problem and a personal and socioeconomic challenge for individuals, families and society (Blumenthal et al., 2011). Population growth linked to unintended pregnancies also represents a serious social problem and a hindrance to improvement of living conditions in less developed regions of the world. Non-use of available contraceptives is responsible for 90% of these unwanted births. A recent report from the
Guttmacher Institute concluded that unmet contraceptive need ranges from 10%–12% in developing countries and that health effects and inconvenience are the main reasons for this non-use (Sedgh et al., 2007). For many women, existing contraceptives are therefore not acceptable or not available and thus in many cases are not used. This prevailing situation highlights the fact that the adoption of a contraceptive method relies not only on access to the available products but also on the product’s acceptability and couple’s willingness and ability to use them effectively. Therefore, it is important to develop new pregnancy prevention options that improve on currently available methods with respect to efficacy, safety, reversibility, and cost-effectiveness, and, which have important public health dimensions (Saha et al., 2010).

Sexually transmitted infections (STIs)

Sexually transmitted infections (STIs) are diseases passed on from one person to another through unprotected sex or sometimes through genital contact. STIs are among the most common infections in adults.
**STI pathogens**

**Bacterial**
- Chancroid (*Haemophilus ducreyi*)
- Chlamydia (*Chlamydia trachomatis*)
- Granuloma inguinale or (*Klebsiella granulomatis*)
- Gonorrhea (*Neisseria gonorrhoeae*)
- Syphilis (*Treponema pallidum*)

**Viral**
- Viral hepatitis (Hepatitis B virus)
- Herpes simplex (Herpes simplex virus 1, 2)
- HIV (*Human Immunodeficiency Virus*)
- HPV (*Human Papillomavirus*)
- Molluscum contagiosum (molluscum contagiosum virus MCV)

**Parasites**
- Crab louse (*Pthirus pubis*)
- Scabies (*Sarcoptes scabiei*)

**Fungal**
- Candidiasis (yeast infection)

**Protozoal**
- Trichomoniasis (*Trichomonas vaginalis*)

**Statistics of STIs**

There are more than 30 bacterial, viral and parasitic pathogens that can be transmitted sexually. If untreated, a number of them can lead to serious complications and sequelae. STIs are a major public-health problem worldwide. Direct treatment costs and serious collateral perinatal damage caused by STIs represent hefty financial and social burdens, particularly in developing countries (Prestholt *et al.*, 2006). The World Health Organization estimates 448 million new cases of bacterial (gonorrheal, syphilitic, and chlamydial infection) and protozoan (trichomoniasis) STIs per year with in each WHO region (WHO,
Globally there are approximately 39.4 million adults and children suffering from HIV infection and, approximately 7,000 new cases of HIV infection are reported worldwide everyday, among which 50 per cent are women. The surveillance programme run by National AIDS Control Organization (NACO) and latest UNAIDS estimate revealed that approximately 2.5 million people in India are seropositive for HIV, which accounts for roughly half of Asia's HIV prevalence (Geneva UNAIDS/World Health Organization, 2010).

The annual incidence of the four major infections per 1000 females in 2005 within each WHO region (Source Geneva: WHO, 2012).

The annual incidence of the four major infections per 1000 males in 2005 within each WHO region (Source Geneva : WHO, 2012).

<table>
<thead>
<tr>
<th>WHO region</th>
<th>Incidence per 1000</th>
<th>New cases (in millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>African Region</td>
<td>130.74</td>
<td>311.83</td>
</tr>
<tr>
<td>Region of the Americas</td>
<td>119.55</td>
<td>118.83</td>
</tr>
<tr>
<td>South-East Asia Region</td>
<td>40.30</td>
<td>45.53</td>
</tr>
<tr>
<td>Eastern Mediterranean Region</td>
<td>44.78</td>
<td>48.23</td>
</tr>
<tr>
<td>European Region</td>
<td>55.60</td>
<td>52.01</td>
</tr>
<tr>
<td>Western Pacific Region</td>
<td>33.73</td>
<td>41.00</td>
</tr>
<tr>
<td><strong>Global total</strong></td>
<td><strong>62.98</strong></td>
<td><strong>82.21</strong></td>
</tr>
</tbody>
</table>


It is estimated that in 2005 there were approximately:

- 101 million new cases of *C. trachomatis*
- 88 million new cases of *N. gonorrhoeae*
- 11 million new cases of syphilis
- 248 million new cases of *T. vaginalis*

This gave a global total of 448 million new cases as shown in Table.

**Womens are at higher risk**

Women are particularly vulnerable to STIs for a variety of intersecting biological, social and cultural reasons. Principle among these is the large mucosal surface area of the female genital tract which is exposed to infected inocula during unprotected sex. In men, the foreskin is the major site of STIs, which has a comparatively smaller surface area, thus reducing vulnerability to
infection (Hladik & Hope, 2009). Women are also made vulnerable to HIV infection by cultural proscriptions limiting their control over when and how they have sex, their knowledge about safe sex practices, their access to condoms, and their ability to negotiate their use (Ramjee et al., 2008). There is thus a clear need for a focus on STIs prevention methods for women, which include development and provision of woman-initiated biomedical means for reducing vulnerability to infection (Ramjee, 2011).

**Vaginal ecology: female genital tract naturally protects against STIs**

Epithelial cells lining the mucosal surfaces of the female genital tract provide the first line of defense against sexually transmitted pathogens such as HIV-1 (Kaushic et al., 2010). Epithelial cells also produce several biological factors, such as defensin, lactoferrin and secretory leukocyte protease inhibitor (SLPI) that have anti-HIV properties (Tomescu et al., 2011). The multilayered squamous epithelium in the vagina and ectocervix provides the first line of defense against HIV. An antimicrobial peptide that contributes to mucosal immunity is secretory leukocyte protease inhibitor (SLPI). SLPI is a small anti-inflammatory protein present in genital tract secretions, semen, saliva, and breast milk. It also possesses potent anti-HIV-1 activity at physiological concentrations. SLPI’s dual role as a mediator of innate immunity and as an anti-inflammatory suggests that conditions or microbicides that trigger a loss in SLPI might increase the risk of STIs acquisition (Herold et al., 2011). Disruption of this barrier enhances acquisition, as illustrated by the increased risk of HIV in the setting of cervical ectopy or genital ulcerative diseases (Ghosh et al., 2010). The vaginal epithelium forms an uninterrupted mucosal barrier between the external microenvironment of the vaginal canal and the underlying tissues. Exposure of the vaginal-ectocervical tissue to chemical insult may cause damage and/or inflammation at the site of application. The public health risk caused by such reactions is significant since increased infection rates for sexually transmitted infections (STI) such as HIV-1 can occur (Fichorova RN, 2004, Weber J et al., 2005). The increased infectivity is due to: a) compromised tissue barrier which allows viral
entry, b) recruitment of susceptible target cells to the site of inflammation (Catalone et al., 2005) and/or c) induction of inflammatory cytokines such as IL-1β and TNF-α which are known to activate HIV LTR via the NFkB pathway (Osborn et al., 1989).

STD/HIV infection through compromised vaginal epithelium and proposed defense strategies. (Source: A Report by the Science Working Group of the Microbicide Initiative).

Normal vaginal flora: Lactobacillus

The microbial species that inhabit the vaginal tract play an important role in the maintenance of health and prevention of infection. The number of microbial species inhabiting the vagina amount to 50. Microbial flora of a healthy premenopausal woman is generally dominated by the Lactobacillus species. Factors such as hormonal changes (particularly estrogen), vaginal pH, and glycogen content can affect the colonization of the Lactobacilli in the vagina (Waigankar and Patel, 2011). In a normal, healthy vagina the pH is generally below 4.5, which largely restricts the types of organisms that can survive. Understandably, acidophilic species of bacteria, such as lactobacilli, typically predominate. Indeed, they are the most commonly isolated organisms from the vaginal microbiota of healthy women and numerous studies have demonstrated that in addition to competing for nutrients, space and host receptors within the vagina, lactobacilli produce
substances detrimental to the growth, survival and colonization of uropathogens. These substances include organic acids, biosurfactants, hydrogen peroxide and bacteriocins, and have been shown to function through pH effects, receptor interference and direct killing (Cadieux et al., 2009). Lactobacilli maintain the ecological equilibrium of the tract by protecting against pathogenic microorganisms (Pascual et al., 2010).

**Dual protection through Spermicidal Microbicides: A viable solution**

**Why dual protection?**

Currently, more than 120 million couples have an unmet need for contraception and over 80 million women have unwanted or unintended pregnancies, 45 million of which are terminated often by unsafe abortions that cause ~68000 deaths annually (Glasier et al., 2006). On the other hand the HIV pandemic continues to haunt the world with ~30 million AIDS deaths reported thus far. The decline in oral contraceptive and diaphragm use and an increase in reliance on condoms and spermicides during recent times suggest that concerns about HIV and other STDs are changing patterns of contraceptive usage among sexually active adolescent and adult women worldwide (Uckun and D'Cruz, 1999). This indicates that in future women would depend heavily on spermicidal microbicides for ultimate safety during heterosexual contacts. Nonoxynol-9 (N-9), the strong surfactant spermicide found in most of the vaginal contraceptives and some sexual lubricants, has been shown to cause vaginal/cervical irritation, inflammation and ulceration, resulting in increased susceptibility of users to HIV infection (Stephenson, 2000, Van Damme et al., 2002). The WHO has cautioned the use of N-9 as a microbicidal spermicide by individuals, especially those who are at a high risk of acquiring STDs/HIV. Since a non-surfactant spermicide is not available to replace N-9 (and other detergents) in vaginal contraceptive preparations, there is an urgent need for a novel and potent spermicidal microbicide.
Targets for dual protection

Human sperm for contraception

Sperm, the male gamete, is a highly specialized cell composed of a head, a short neck, and a long tail, intended for the task of fertilizing an egg. The head contains the haploid nucleus in a highly compact form and the acrosome, a specialized secretary vesicle with hydrolytic enzymes, whereas the tail is a long flagellum that is composed of a middle piece, principal piece, and end piece. The tail contains the motile apparatus necessary for the movement and penetration of sperm into the egg at fertilization. Motility is generated by a highly organized microtubule-based structure called the axoneme that is surrounded by outer dense fibers in the middle and principal pieces of the tail. A mitochondria sheath in the middle piece and a fibrous sheath in the principal piece cover the outer dense fibers.

Conception is defined as the fusion of an egg cell with a sperm cell. Methods which enable sexual intercourse without fusion of egg and sperm, or with reduced risk of pregnancy, are defined as methods of pregnancy prevention or contraception. The methods for pregnancy prevention may be divided into
methods which use hormones and those which do not. Pregnancy prevention methods with the use of hormones may be divided into combined estrogen-progestogen contraceptives (pills, skin patches, and vaginal rings), progestogen-only contraceptives (pills, injections, implants, hormone spirals) and emergency contraceptives. Pregnancy prevention methods which do not use hormones are methods without the use of any appliances (abstinence and discontinuation of sexual intercourse), chemical contraceptives (spermicides), mechanical contraceptives (condoms, spirals, and vaginal rings), surgical contraception (tubal ligation, vasectomy) as well as simultaneous combination of several methods (Gorenoi et al., 2007). In the general population, hormonal contraceptive use has been associated with an increased risk of metabolic dysregulation, especially lower serum high density lipoprotein (HDL) concentrations, hypertriglyceridemia, insulin resistance, and elevated serum glucose concentrations (Womack et al., 2009). The use of hormonal contraception may also elevate the risk of HIV acquisition, in some instances dramatically (Heffron et al., 2012). The relative risk of thrombosis on combined oral contraception is three to fivefold in users in comparison with non-users (Trenor et al., 2011). The use of OCPs and IUDs is becoming restricted the world over due to increased awareness of the associated side-effects. Condoms provide adequate dual protection (McMahon et al., 2004) but are not controlled by women or convenient, besides having problems of acceptability and disposal. It would be quite logical to believe that a method of pregnancy protection (other than condom) might have been employed in many of those heterosexual contacts that ultimately resulted in an HIV/STD infection (Stone & Jiang, 2006). Topical applications (such as vaginal gels) with microbicidal and spermicidal activity that can be used by women without the need for consent of a male partner are one possible answer to the problem (Inacio et al., 2011). Thus, use of a pure microbicide would be limited if it does not offer the additional advantage of pregnancy protection. Accordingly, a potent contraceptive (spermicidal) activity in microbicides would attract users, especially those at a high risk of acquiring HIV/STD, and may improve compliance in usage (Sieving et al., 2007).
**Trichomonas vaginalis**

**Complete Classification:**

Domain: Eukarya (larger, more complex cell)

Kingdom: Protista (difficult to classify eukaryotic organisms, most diverse).

Phylum: Metamonada (flagellated protozoa)

Class: Parabasilia (have modified mitochondria, have one or more flagella)

Order: Trichomonadida (have undulating membrane, axostyle)

Genus: *Trichomonas* (affect not only people, but poultry and other farm animals)

Species: *Trichomonas vaginalis* (cause infection in vagina)

*T. vaginalis* is a single-celled, teardrop shaped protist that can grow up to 5 and 20 µ in width. Four anterior and one posterior flagella allow *T. vaginalis* to move. The posterior flagellum is part of the center axostyle, and has a barbwire-like structure. This structure allows the protist to attach to and tear the urethra or vaginal walls, which causes inflammation and aids in speeding and intensifying infection. *T. vaginalis* has a cell membrane, but lacks a cell wall. (Arroyo *et al.*, 1993; Warton, 1979). The flagella and the undulating membrane give this parasite a characteristic quivering motility (Honigberg *et al.*, 1964). Under unfavorable growth conditions, *T. vaginalis* can round up and internalize the flagella. Some believe these forms to be pseudocysts, but it is more likely that
they are degenerate forms of *T. vaginalis*, since they have not been reported to give rise to normal motile forms (Fari *et al.*, 1985; Honigberg and Brugerolle, 1990). A visible nucleus is located at the organism's center. *T. vaginalis* lacks traditional mitochondria, like all other excavates. In place of true mitochondria, there is an anaerobic form of mitochondria present in these parasites known as a hydrogenosome (Mary *et al.*, 2011).

**Why *Trichomonas vaginalis***?

**Trichomoniasis: The most common STI**

Trichomoniasis, caused by *Trichomonas vaginalis*, is one of the most common nonviral sexually transmitted diseases (STDs): annually, ~248 million infections occur worldwide (WHO, 2011). Trichomoniasis is a frequent cause of vaginitis and can contribute to premature rupture of membranes during pregnancy, preterm birth, low birth weight, and may facilitate HIV acquisition (Robert *et al.*, 2012). *T. vaginalis* colonizes the female and male urogenital tract and symptoms vary widely from asymptomatic infection to acute vaginitis, urethritis and prostatitis (Mukaida, 2000). In women it may cause adverse pregnancy outcomes like low birth weight, preterm delivery (Gilbert *et al.*, 2000), premature rupture of membranes, infertility (Ferguson, 1999, Singh *et al.*, 2004, Sorvillo *et al.*, 2001), predisposition to cervical cancer (Zhang and Begg, 1994, Yap *et al.*, 1995, Zhang *et al.*, 1995, Afzan at al., 2012) and increased susceptibility to HIV/AIDS (Wasserheit, 1992, Laga *et al.*, 1993, Sorvillo and Kerndt, 1998, Sorvillo *et al.*, 2001). Among other sequels of trichomonosis are orchitis associated with oligoasthenoteratospermia and hypogonadism (Lloyd *et al.*, 2003), newborn urinary tract infections with chronic lung disease (Hoffman *et al.*, 2003). The most serious consequence of trichomoniasis is that it expands the portal of viral exit from HIV-positive patients and viral entry in HIV-negative patients (Sorvillo *et al.*, 2001). Hence HIV-positive men with symptomatic trichomoniasis have higher concentrations of infectious HIV in semen (Hobbs *et al.*, 1999), while HIV-negative women with trichomoniasis are highly susceptible to seminal HIV infection. This facilitates man-to-woman HIV transmission. It is estimated that ~24% of HIV infections are directly attributable to *T. vaginalis* infections (Mundodi *et al.*, 2004). Therefore, control of this STD
alone may prove to be one of the most effective means of managing the HIV-transmission risk worldwide (Soper, 2004).

**Relation of T. vaginalis with HIV & other STIs**

There is a strong association between bacterial and viral sexually transmitted infections and both the acquisition and transmission of HIV infection (Ward & Rönn, 2010). Prospective studies strengthened this observation by showing a link between STI and incident HIV infection, with the strongest relative risks for genital ulcer disease but potentially large attributable risks from more common inflammatory conditions such as trichomoniasis (Cameron et al., 1989). *T. vaginalis* infection is highly associated with the presence of other sexually transmitted infections, including gonorrhea, Chlamydia, and sexually transmitted viruses. *T. vaginalis* infection increases the susceptibility to pathogenic viruses, including herpes, human papillomavirus (HPV), and HIV (Huppert, 2009). Persons with trichomoniasis are twice as likely to develop HIV infection as the general population (Laga et al., 1993). Two explanations exist for the association between *T. vaginalis* and HIV, (a) disruption of the epithelial monolayer leading to increased passage of the HIV virus and (b) induction of immune activation, specifically lymphocyte activation and replication and cytokine production, leading to increased viral replication in HIV-infected cells. There are several biological mechanisms thought to account for the synergy between HIV and STI epidemics. Infections that disrupt the epithelial surface of the genital tract may increase acquisition through facilitating the access of HIV-1 to target cells under epithelial surface thus increasing the probability that HIV-1 is able to establish a systemic infection (Fox & Fidler, 2010). Pregnant women with *T. vaginalis* infection are more likely than uninfected women to deliver preterm or to have other adverse pregnancy outcomes, including low birth weight, premature rupture of membranes, and intrauterine infection. (Forna et al., 2003). *T. vaginalis* infection may also increase the transmission of HIV owing to a disruption of the vaginal mucosa and respiratory or genital infection in the newborns (CDC, 2006). One study reported a higher risk of pelvic inflammatory disease in women with trichomoniasis (Moodley et al., 2002). Other studies have
reported a 1.9-fold risk of tubal infertility in women with trichomoniasis (Grodstein et al., 1993). Trichomoniasis may also play a role in cervical neoplasia and postoperative infections. In men, complications of untreated trichomoniasis include prostatitis, epididymitis, urethral stricture disease, and infertility. Infertility may result from decreased sperm motility and viability (Soper, 2004).

**Spermicides: Stopping sperm at the gate**

Spermicides are chemical agents that immobilize/kill the spermatozoa in the vagina and exert their contraceptive effect in the female genital tract. Spermatozoa have been found in the cervical mucus within 90 s after coitus. To effectively prevent sperm migration, the spermicidal agent would have to act rapidly to prevent the penetration of spermatozoa into the endocervical canal as well as attack sperm residing in the vagina for a longer period of time (Bernstein, 1974). The failure of a vaginal contraceptive is often due to mistakes in the application of spermicide rather than its poor contraceptive efficacy. To be an effective contraceptive agent, a spermicide must act rapidly and efficiently to immobilize (kill) sperm on contact, have good cervical mucus biodiffusion, and render the spermatozoa totally incapable of fertilization. It also needs to be nonirritating to the vaginal/cervical and penile mucosa, should not have an adverse effect on the developing embryo or fetus and must be free of long-term toxicity, moreover it should also be systemically nontoxic (Lee, 1996).

**Microbicides: Stopping HIV/STIs at the gate**

Microbicides are products that are being developed to reduce the transmission of HIV, and possibly other sexually transmitted infections, when used intra vaginally during intercourse. They can be formulated as gels, creams, films, or suppositories. Microbicides may or may not have spermicidal activity (contraceptive effect). According to the Joint United Nations Programme on HIV/AIDS (UNAIDS, 2007), approximately 40 million adults worldwide were living with HIV and AIDS at the end of 2006, including 4.3 million who were newly infected. UNAIDS also reports that ~45 million new HIV infections
occurred between 2002 and 2010. The majority of new HIV infections occur through heterosexual intercourse. Women are increasingly affected by HIV and AIDS; by the end of 2006 there were an estimated 17.7 million women worldwide who were HIV-positive, an increase of 1.2 million women in two years (UNAIDS, 2007). Microbicides can be used by women without negotiation with their partners. This is an important characteristic because condom-dependant protection has been unsuccessful in halting the spread of HIV and other sexually transmitted infections.

**Vaginal microbicide/contraceptive products in the market/under clinical trials**

**Anti-Trichomonal drugs and drug resistance**

Treatment for trichomoniasis has relied almost exclusively on drugs of the 5-nitroimidazole class. In the USA, trichomoniasis is treated with either metronidazole or tinidazole. Tinidazole has better molar efficacy against *T. vaginalis* isolates in vitro (McClelland, 2008) and has fewer side effects than metronidazole. However, because of the similarities in chemical structure, infections that are highly resistant to metronidazole may also fail to cure after standard treatments with tinidazole. Both the action of the 5-nitroimidazoles and the specific mechanism of resistance in *T. vaginalis* isolates to these drugs remain poorly understood. In general, 5-nitroimidazoles enter the parasite by passive diffusion as a prodrug. Activation of the drug occurs within the parasite by redox enzymes involved in ATP production in the hydrogenosome and/or by flavin reductases, such as thioredoxin reductase (Leitsch et al., 2010). Reduction of 5-nitroimidazoles produces cytotoxic radical anions that damage DNA and form adducts with critical parasite proteins. Resistance of *T. vaginalis* to 5-nitroimidazoles is quantitative and not qualitative; thus, infections that fail to cure with standard treatment doses can often be cleared with higher, more prolonged treatment with the same drugs (Bosserman et al., 2011). This is clearly not an ideal strategy for dealing with drug-insensitive infections and may result in selection of more highly resistant strains. Alternative treatments for
trichomoniasis utilize compounds that are not absorbed well from the intestinal tract (paromomycin sulfate, furazolidone) or are not ingestible (povidone iodine) and therefore must be administered intravaginally. While these compounds are very effective against trichomonads in vitro, intravaginal therapy tends to be less efficacious than systemic treatment in contacting and killing all parasites. In non-pregnant women, metronidazole is the approved US-FDA drug against trichomoniasis by oral route, but is less effective when used vaginally. Also, its non-spermicidal nature makes it ineffective as a vaginal contraceptive microbicide. On the other hand, some anti-trichomonal drugs like emetine, quinine and quinacrine have a rather moderate spermicidal activity.

Some drug structures having promising activity against T. vaginalis.

Spermicides/microbicides/devices under clinical trials

The concept of a topical vaginally applied “virucide”, the application of which would be controlled by women themselves, was mooted in 1990 by Zena Stein (Stein, 1990). Several types of such topical vaginally applied gels (later referred
A list of virtually every method currently proposed or under active investigation as an option to interrupt the unintended pregnancies and transmission of HIV/STIs. (Source: A Report by the Science Working Group of the Microbicide Initiative).

No lead molecules are currently under investigation for this mechanism of action (but see further discussion of DC-SIGN in the following section).

**Multiple mechanisms**

**Antiviral proteins**
- MAP30 (Mamorinda Anti-HIV Protein)
- GAP31 (Gelonin Anti-HIV Protein)

**Other molecules**
- cellulose acetate phthalate (CAP) (virus activation and/or entry inhibitors)
- monocapsin (HIV inactivation)
- PAVAS (viral binding inhibition)
- neptalbumin (HIV neutralization)

**Thermoreversible gels**
- a physicochemical barrier that acts as a double barrier—(i) physical barrier, the gel by itself; and (ii) chemical barrier, sodium lauryl sulphate, for viral inactivation
- invisible Concom®

**Uncharacterized agents**

**Viral disruption**
- Oxidants
  - povidone iodine
  - oxidative chlorine
  - haloperoxides
  - chlorhexidine

**Surfactants**
- C-31G (Savvy®)
- nonoxynol-9 (various formulas)
- octoxynol-9 (Gestra Plus®)
- benzalkonium chloride (BZK)
- acyl glycerol/milk lipids
- polyglycosides
- sodium dodecyl sulphate (SDS)
- Z-14 (acyl-carnitine analogue / hemocyanin and related lipids)

**Attachment or fusion inhibitors**

**Sulfated and other charged polymers**
- carrageenan (PC-515/ Carraguard®)
- cellulose sulfate (CS/Ushereel®)
- sulfated carbohydrate polymer (PGR-2000®)
- doxyl-2-sulfate (DS/Emmetal®)
- heparoid sulfate/cholic acid
- polyanionic gel120 inhibitors
- polystyrene sulfonate (PSS)

**Fusion inhibitors**
- cyanovirin-N
- trimetoprim (TSP)-1 derived peptides
- bococirculin (CD)
- chimeric protein with soluble CD4
- CCR5 inhibitors
- T-20

**Inhibition of reverse transcription**

**Nucleoside and non-nucleoside RTIs**
- zidovudine
- stavudine
- tenofovir (PMPA)
- UC-781
- VHH-07 (an AZT derivative)

A list of virtually every method currently proposed or under active investigation as an option to interrupt the unintended pregnancies and transmission of HIV/STIs.

(Source: A Report by the Science Working Group of the Microbicide Initiative).

to as microbicides) have been tested in the past. These included surfactants, polyanionic entry inhibitors, and acid buffers (Ramjee et al., 2010). A number of vaginal products that offer STI/HIV protection with or without protection against pregnancy are under thorough study. Acid buffers acidify the semen in the vagina for killing the spermatozoa and maintain the natural protective
microbicidal acidity of the vagina in the presence of alkaline semen. **BufferGel** (ReProtect LLC, Baltimore, MD) is a very effective microbicidal spermicide in animals and humans. **ACIDFORM** is also formulated to form a protective layer over vaginal and cervical epithelium with long-term vaginal retention. **Bromomethoxy zidovudine derivatives** offer another class of safe and effective dualfunction microbicidal spermicides. **Thiourea derivatives** are also being considered as promising dualfunction microbicidal spermicides with a potent HIV-1 reverse-transcriptase inhibitory activity. There are three polyanionic entry inhibitors in clinical trials ie; **Carraguard, PRO 2000** and **cellulose sulphate**.

**Failure of some vaginal products in clinical efficacy trials**

Surfactant-based devices for purposes of contraception have been in use since decades and the first microbicidal vaginal gels to be tested in clinical trials were surfactant-based. However, all the surfactant-based microbicide candidates that completed phase III clinical trials failed to prevent HIV infection and their utility as general microbicides was also questionable. The first to fail was **nonoxynol-9**. The failure of N-9 was followed by C31G, a mixture of a zwitterionic and a nonionic surfactant, in a formulation known as **“SAVVY Vaginal Gel”**. More recent phase III studies, with nonspecific (broadly acting) microbicide gels that did not include surfactants (**Carraguard and PRO 2000**) also did not demonstrate efficacy. Carraguard and PRO 2000 are considered non-specific microbicidal gels (Romano et al., 2012) as these are rather broad-spectrum microbicidal compounds that act as non-specific anti-viral and anti-bacterial agents (somewhat like nonoxynol-9) against HIV and a variety of other STD-pathogens. The action of the active compound in these drugs is not restricted to any particular type of STD pathogen. Carraguard and PRO 2000 were found to be safe for use, but did not have any effect on HIV acquisition. Phase III study of the cellulose sulfate has been stopped prematurely because of a higher number of HIV infections in the active compared with the placebo group. Unfortunately, several earlier microbicide trials yielded disappointing results, highlighting the complexities of microbicide development. Not only did the products fail to protect against HIV, but also several (**N-9, C31G** (Savvy) and **cellulose sulfate** were associated with higher rates of HIV infection. Subsequent studies provided
insights into the biological mechanisms that likely contributed to the increased risk for HIV including disruption of the epithelial barrier, induction of an inflammatory response, and loss of protective antimicrobial peptides (Herold et al., 2011). None of the previous generations were able to prevent the acquisition of HIV infection by women, and two (N9 and cellulose sulphate) may have

<table>
<thead>
<tr>
<th>Study/Sponsor</th>
<th>Microbicides</th>
<th>Study Outcome</th>
<th>Potential Biological Mechanisms Contributing to Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL-1492</td>
<td>N-9</td>
<td>↑ HIV acquisition risk</td>
<td>Disruption of epithelial barrier</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inflammation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↓ SLPI</td>
</tr>
<tr>
<td>CONRAD</td>
<td>Cellulose sulfate</td>
<td>Stopped when interim analysis indicated ↑ risk of HIV in treatment arm</td>
<td>↓ drug activity when Virus introduced in semen</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>disruption of epithelial barrier</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>high EC50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↓ SLPI</td>
</tr>
<tr>
<td>Family Health International</td>
<td>Cellulose sulfate</td>
<td>Stopped based on CONRAD study results</td>
<td>Surfactant</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Disruption of epithelial barrier</td>
</tr>
<tr>
<td>Family Health International</td>
<td>C31G</td>
<td>Trend towards ↑ HIV risk</td>
<td>↓ activity against R5 isolates</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stopped for futility</td>
<td>↓ activity in presence of semen</td>
</tr>
<tr>
<td>Population Council</td>
<td>Carraguard</td>
<td>Safe but ineffective</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPTN035</td>
<td>PRO 2000 BufferGel</td>
<td>Safe but ineffective</td>
<td>PRO 2000: ↓ activity in presence of semen</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>loss of drug following sexual intercourse</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↓ SLPI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BufferGel: Insufficient acidbuffering activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>following exposure to semen; HIV isolates acid-resistant</td>
</tr>
</tbody>
</table>

Clinical Microbicide Efficacy Trials: Outcomes and Biological Mechanisms Potentially Underlying Results. (Herold et al., 2011).
Current microbicides in clinical trials\textsuperscript{a}

<table>
<thead>
<tr>
<th>Product\textsuperscript{b}</th>
<th>Mechanism of action</th>
<th>Phase</th>
<th>In vitro susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>BufferGel</td>
<td>Vaginal defense enhancer</td>
<td>II/III</td>
<td>HIV 1 (cell free), <em>Treponema pallidum</em>, HSV, CT, <em>H. ducreyi</em>, NG, BV-associated bacteria, <em>S. aureus</em>, sperm, <em>T. vaginalis</em>, <em>E. coli</em></td>
</tr>
<tr>
<td>Acidform</td>
<td>Vaginal defense enhancer</td>
<td>III</td>
<td>Results not available</td>
</tr>
<tr>
<td>BufferGel Duet</td>
<td>Vaginal defense enhancer</td>
<td>I</td>
<td>Results not available</td>
</tr>
<tr>
<td>Invisible Condom</td>
<td>Entry/fusion inhibitor</td>
<td>I/II</td>
<td>HIV-1 (cell-free and cell-associated), HSV, HPV, CT, NG, sperm</td>
</tr>
<tr>
<td>PRO 2000/5 Gels</td>
<td>Entry/fusion inhibitor</td>
<td>III</td>
<td>HIV-1 (cell-free and cell-associated), HSV, CT, NG, sperm</td>
</tr>
<tr>
<td>VivaGel</td>
<td>Entry/fusion inhibitor</td>
<td>I</td>
<td>HIV-1 (cell-free and cell-associated), HSV</td>
</tr>
<tr>
<td>Tenofovir/PMPA Gel</td>
<td>Replication inhibitor</td>
<td>II/IIB</td>
<td>None found</td>
</tr>
<tr>
<td>UC-781 Gel</td>
<td>Replication inhibitor</td>
<td>I</td>
<td>HIV-1 (cell-free and cell-associated)</td>
</tr>
<tr>
<td>TMC 120 Vaginal ring and gel</td>
<td>Replication inhibitor</td>
<td>I/II</td>
<td>HIV-1 (cell-free and cell-associated), <em>Lactobacillus crispatus</em>, NG, <em>H. ducreyi</em>, HSV, HPV</td>
</tr>
<tr>
<td>PC 815</td>
<td>Replication inhibitor</td>
<td>I</td>
<td>Not available</td>
</tr>
<tr>
<td>Praneem</td>
<td>Replication inhibitor</td>
<td>III</td>
<td>HIV-1 (cell-free and cell-associated), HSV, CT, NG, sperm</td>
</tr>
</tbody>
</table>

HSV, herpes simplex virus; CT, *C. trachomatis*; NG, *N. gonorrhoeae*; BV, bacterial vaginosis; HPV, human papillomavirus

\textsuperscript{a} From Alliance for Microbicide Development website updated July 2008 (http://www.microbicide.org)

\textsuperscript{b} Manufacturers: BufferGel: ReProtect; Acidiform: Instead; BufferGel Duet: ReProtect; Invisible Condom: Université Laval; PRO 2000/5 Gels: Indevus Pharmaceuticals; VivaGel: Starpharma; Tenofovir/PMPA Gel: Gilead Sciences, Forester City, CA, USA; UC-781 Gel: CONRAD, Arlington, VA, USA; TMC 120 Vaginal ring and gel: International Partnership for Microbicides, Silver Spring, MD, USA; PC 815: Population Council; Praneem: Talwer Research Foundation, New Delhi, India.
somewhat unexpectedly increased susceptibility to infection through damaging effects on the vaginal epithelium (Karim et al., 2011., Mesquita et al., 2009) which may have been caused by very frequent use of the product.

There are however, a few successes stories that have been reported recently with anti retro-viral (ARV) based prevention strategies, e.g. the CAPRISA 004 and TRUVADA trials. The Centre for the AIDS Program of Research in South Africa (CAPRISA) 004 trial assessed the effectiveness and safety of a 1% vaginal gel formulation containing tenofovir, a nucleotide reverse transcriptase inhibitor, for the prevention of HIV acquisition by women. The gel was found 39% effective in reducing the risk of contracting HIV during sex, and, 51% effective in preventing genital herpes infections (Karim et al., 2010). On the other hand daily intake of TRUVADA, a fixed-dose combination of two anti-retroviral drugs: EMTRIVA® (200 mg emtricitabine) and VIREAD® (300 mg tenofovir disoproxil fumarate), achieved 99% reduction in risk of contracting HIV in high risk individuals (Collins., 2012). The Food and Drug Administration approved it for prophylactic use on July 16, 2012 (Perrone., 2012). Results of microbicide clinical trials turned the main focus of current research to the development of formulations containing novel, preferably specifically acting molecules.

Nonoxynol-9: The approved spermicide - failed as microbicide

Nonoxynol-9 is a surfactant microbicide that has been used as a spermicide for more than half a century. It acts by disrupting the cell membrane of sperm as well as those of some sexually transmitted viral and bacterial pathogens (Jain et al., 2005). N-9 is the active ingredient in most over-the-counter birth control products available in the United States, Canada and Europe. Contraceptive jellies and creams (such as those used with the diaphragm), Conceptrol suppositories, Delfen foam, Vaginal Contraceptive Film (VCF), Encare Oval suppositories and Advantage-S gel are all products that contain N-9. The mechanism of spermicidal action of these products involves disruption of the sperm's outer membrane and cell death. N-9 products were designed and approved by the FDA as contraceptives, not as microbicides. But, since N-9 kills HIV and a wide
variety of other STI pathogens in the test tube, the idea of using it for disease prevention has been around for decades. Recent evidence, however, has called into question even modest claims of protection. Three randomized controlled trials have failed to detect any statistically significant effect of N-9 against common bacterial STIs (Roddy et al., 1998; Van Damme, 2000; Roddy et al., 2002). As a result, the US Centers for Disease Control and the World Health Organization have concluded that products containing N-9 should not be promoted for STI protection. Furthermore, N-9 products used in clinical trials not only failed to offer any protection against STDs and HIV, but on the contrary, they actually increased the incidence of these diseases in the users (Roddy et al., 2002; Wilkinson, 2002; Stephenson, 2000; Forbes & Heise, 2000). In a recent report, the WHO has cautioned the use of N-9 for STD and HIV protection. The strong surfactant nature of N-9 has shown to cause vaginal irritation and lesions in repeated use resulting in increased susceptibility to STD pathogens (Wong et al., 2002; WHO, 2002).

**Some facts about Nonoxynol-9 and other detergent spermicides**

At present all commercially available spermicidal microbicides have detergent ingredients that disrupt cell membranes. These include the surfactants Nonoxynol-9, octoxynol-9. Worldwide, the cationic surfactant benzalkoniumchloride, the anionic detergent sodium docusate are also used as vaginal spermicides. N-9 is only about 75% effective in preventing pregnancy (Trussell and Trost, 1987; Kulig et al., 1989; Raymond and Dominik, 1999). Additionally, N-9 is only marginally effective in preventing gonococcal and chlamydial cervical infections respectively (Cook and Rosenberg et al., 1998). Thus the dual protection provided by N-9 if any, is nominal. The spermicidal activities of these surfactants are associated with their structural affinity to the membrane lipids (Schill and Wolf, 1981; Wilborn et al., 1983). Major drawback of using N-9 or other surfactants is their detergent type effect on epithelial cells and normal vaginal flora. Frequent use of N-9 has been associated with an increase risk of vaginal or cervical infection, irritation or ulceration (Niruthisard et al., 1991; Rekart, 1992; Roddy et al., 1993; Weir et al., 1995). Detergent type spermicides alter vaginal flora and lead to an increase in opportunistic infections.
Such opportunistic infections are known to enhance the susceptibility of the cervicovaginal epithelium to HIV infection (Augenbraun and McCormack, 1994). N-9 is rated as a class 2-3 toxic substance (Kreiss et al., 1992). N-9 displays antiviral and spermicidal activities only at cytotoxic doses (D’Cruz et al., 1999). Therefore, new, effective, safe and female-controlled topical dual-action microbicide lacking detergent-type membrane toxicity should have clinical advantages over the currently available vaginal microbicides. The development formulations with multiple activities (dual contraceptive and anti-HIV/STIs activity) will provide women with an expanded range of mechanisms for ensuring their own health and that of their families (Ramjee, 2011).

Safety issue with vaginal products
Of utmost importance in the development and evaluation of topical microbicides is safety with recent efforts focused on the action of such agents on epithelial integrity. Effective preclinical microbicide safety testing is of great importance to insure that only the most promising candidates are advanced to clinical trials. This is particularly important in light of the fact that several clinical trials of emerging microbicides have been terminated due to safety concerns (Vargas et al., 2012). Among these agents is nonoxynol-9, which initially was shown to have effective antimicrobial activity against a number of STI agents in vitro, including human immune-deficiency virus (Malkovsky et al., 1988). However, N-9 failed to act as an effective microbicide in a clinical trial (Roddy et al., 1998), and in two efficacy trials frequent use was linked to an increase in the incidence of HIV infection (Van et al., 2002). A trial of another microbicide candidate cellulose sulfate was also recently terminated in light of safety concerns (Ramjee, 2007). These events have provided the impetus for an increased emphasis on new methods for preclinical assessment of microbicide safety.
Urgent need of safe and dually-functional novel preparations

Young women are at greatest risk for unintended pregnancy and contracting a variety of sexually transmitted infections (STIs). For over a decade, medical practitioners, researchers, and professional organizations have recommended dual protection strategies designed to enhance prevention of both unintended pregnancy and STI/HIV (Sales et al., 2012). A new generation of dual-function spermicides that offer protection against STIs (including HIV) and pregnancy and whose action is mechanism-based are urgently needed by the society (Trager, 2003; Uckun & D'Cruz, 1999). This need is also augmented by the fact that currently the use of OCPs and IUDs is becoming restricted the world over due to increased awareness of the associated side-effects. The use of vaginal contraceptives as local (topical) agents in a need-based manner (not requiring regular intake/application) makes them relatively much safer. A microbicidal spermicide can also be used as an adjunct to condoms or in medication of condoms to increase their efficacy and safety (Oshio et al., 1990). Thus, an ideal spermicide would be the one that is preferably nondetergent/nonsurfactant action, is non-irritant, has a high aqueous solubility and thus a good cervical mucus biodiffusion, with microbicidal (including anti-HIV) activity, and is also nonhostile to normal vaginal flora.

Combination of microbicides and spermicides: Enhancing efficacy

Combinations expressing synergistic or additive effects may be particularly useful for a variety of reasons, including:

- Maximizing activity, when direct synergy against a specific STI can be demonstrated;
- Decreasing the potential for resistance;
- Increasing the spectrum of activities (anti-STI and spermicidal)
- Reducing the required concentration of expensive or potentially toxic agents.
Protection against several potential STIs as well as unwanted pregnancies is likely to be achieved only by the combination of topical microbicidal and spermicidal agents. Indeed, full protection against HIV alone may also require a combination microbicide in future. The design of broad-spectrum combinations can be rationalized by understanding more fully the biological effects of individual agents, although such combinations will also need to be fully tested to rule out any potential for unanticipated antagonism between agents.

**The Present Study**

In the present study we have addressed the problems of both unwanted pregnancies and sexually transmitted infections and have attempted to identify some novel treatment/management modalities using both designed molecules (modern drug candidates) and natural products from plant source. Topical contraceptive microbicides are now considered to be the last ray of hope, as they would ideally provide protection against unwanted pregnancy, proper lubrication during sexual activity, and preclude the vaginal/rectal transmission of sexually transmitted diseases. The present study is aimed at designing novel prophylactic contraceptive preparations by combining a potent non-detergent, synthetically designed spermicide with a strong natural microbicide in a logical ratio to provide safe and effective ‘microbicidal contraception’. The idea is to offer an option for contraception that is not only more effective and safe than N-9 but also capable of controlling trichomoniasis, the most common STD that significantly increases probability of viral STDs and HIV infection. It is intended to study the contraceptive/microbicidal efficacy of the new formulation(s) *in vitro* as well as *in vivo*. Besides combining spermicide and anti-trichomonas molecules, it is also aimed to design some novel dually capable molecules which could potentially tender anti-Trichomonal contraception. The chemical designing and identification of potent molecules was done in collaboration with the Medicinal and Process Chemistry Division of CDRI. The aims and objectives of the present study could be broadly classified under the following five heads:
• To design novel prophylactic contraceptive(s) using combination of promising spermicidal and microbicidal agents and to evaluate efficacies in vitro
• To establish the safety of the formulation(s) for topical intravaginal application
• To assess the efficacy of formulation in preventing vaginal/cervical inflammation caused by infection with T. vaginalis
• To determine the molecular mechanism of Trichomonas infection and to propose a mechanism for the anti-Trichomonas activity of promising agents/combinations
• To work out a product design in form of a vaginal gel/foam-tablet, utilizing the most promising combination of active ingredients and assess its in vitro efficacy