Present status of Spermicidal agents
Present Status of Spermicidal Agents

Several synthetic and natural products have been studied for their spermicidal activity. Based on their mode of action these can be broadly be classified into following categories:

1. **Electrolytes:** These agents are primarily concerned with the disruption of tonicity of spermatozoa, which results in decreased spermatozoal activity. The prominent examples are boric and tartaric acids, which generate foaming action to exert their spermicidal effect.\(^{18}\) EDTA\(^{19}\) (1), a cationic chelating agent showed spermicidal activity by modulating calcium ion concentration in semen.

![Formula 1](image)

A pharmaceutical preparation containing myristamido propyl dimethyl benzyl ammonium chloride can be used as spermicides, anti-HIV agents or antiseptics.\(^{20}\) Potash Alum solution have different effects on sperm at different concentrations. Ethonium-1,2-ethylene-bis- (N-dimethylcarbdecyloxyethyl)-ammonium chloride (2) has showed very good spermicidal activity.\(^{21}\)

2. **Sulphydryl Binding Agents:** The plasma membrane, head and tail region contain various proteins rich in disulfide linkages. The dynamic conversion of thiols to disulfide linkages forms an essential part of sperm maturation. Interference with these biochemical phenomena could lead to lack of maturity or death of spermatozoa. Sulphydryl binding agents exert their action by oxidation, alkylation or formation of mercaptides. Hydrogen peroxide, o-iodobenzoate and several hydroquinones are known to destroy the tertiary protein structure by converting the thiol group of cysteine to disulphide linkages. Phenyl mercuric acid is another mercaptide forming agent.

![Formula 2](image)

![Formula 3](image)

![Formula 4](image)
Maleimide (3) derivatives have been tested as spermicidal agents by inhibiting sperm motility with affecting vaginal and cervical epithelium integrity.\textsuperscript{22}

Selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine (4) have recently been suggested to immobilise sperm by sulfhydryl binding or inhibition of oxidative phosphorylation.\textsuperscript{23}

3. Bactericides: During the past decade several agents have been screened for their spermatozoa immobilizing properties. This class consisted primarily of bactericides, which are often spermicidal due to their ability to interact with membrane components. Prominent among them are alkoxyln 741,\textsuperscript{24} benzalkonium chloride\textsuperscript{25} (5).

![Bactericide Structure](image)

Piperazine dicarboxamidine\textsuperscript{26} (6) derivatives are very good bactericides, fungicides and spermicides.

![Piperazine Dicarboxamidine](image)

Quaternary ammonium chlorides and octyl phenoxy poly ethoxy ethanol showed good germicidal and spermicidal activities.\textsuperscript{27} Different phospholipids were tested for their antibacterial, antifungal, spermicidal and virucidal activities.\textsuperscript{28} Cellulose sulfate\textsuperscript{29} was reported to show both antimicrobial and spermicidal activities. Acyl Carnitine\textsuperscript{30} analogs showed spermicidal activity along with microbial and anti-HIV activities in \textit{in vitro} studies. Cyclic Carnitine\textsuperscript{31} (7) analogues showed topical microbicidal and spermicidal activities.
Chlorohexidine diacetate and p-nitro phenol have strong spermicidal and antibacterial effects.\textsuperscript{32} C31G,\textsuperscript{33} a mixture of two synthetic amphoteric surface active compounds cetyl betaine and myristamine oxide is a broad-spectrum antibacterial agent that shows contraceptive properties in vitro. Nisin\textsuperscript{34}, a known antimicrobial peptide showed spermicidal activity and could serve as a safe vaginal contraceptive for future therapeutic interventions in STIs. Bactericidal ointment containing aloe and Benzalkonium chloride is used for killing AIDS virus and sperms and prevention of sexual transmitted diseases.\textsuperscript{35} However, acute toxicity and side effects such as itching and burning have limited the scope of these agents. A common disadvantage associated with bactericides includes non-specific nature of action, which tends to disturb the vaginal flora and the long-term use enhances the risk of STD infections.

4. **Surfactants:** Other agents capable of interacting with the lipoprotic membrane of spermatozoa include nonoxynol-9 (8), octoxynol-9 (9), dodecaethylene glycol monolaurate, methoxypolyoxyethylene glycol 550 laurate, menfegol, laureth 10S that have been approved by FDA.\textsuperscript{36}

\[
\begin{align*}
\text{NONOXYNOL-9} & \quad \text{C}_{9}H_{19}-(OCH_{2}CH_{2})_{9}\text{OH} \\
\text{OCTOXYNOL-9} & \quad \text{C}_{8}H_{17}-(OCH_{2}CH_{2})_{9}\text{OH}
\end{align*}
\]

However, only nonoxynol-9 and to a lesser extent octoxynol-9 are commercially exploited spermicides presently available. Though the anti HIV efficacy of nonoxynol-9 has been demonstrated \textit{in vitro}, it has no effect on transmission HIV/AIDS and STD infections.\textsuperscript{37} Furthermore, this is coupled with side effects such as vaginal discomfort, cervicites, restriction of
spermicidal action to vagina and not to cervix, pH dependency, messy nature and inability to maintain a uniform concentration of spermicide for long time which has limited the scope of nonoxynol-9. Two other agents RS 37367 (10) and d-propranolol (11) have been studied for their spermiostatic activity whereas RS 37367 is 50 times more potent than N-9, d-propranolol is less effective than N-9. Both of these suffer from formulation difficulties.  

Alkyl derivatives of polyethoxy phenoxy ethanol were synthesized and used with vaginal films and suppositories. Chlorohexidine showed a promising activity similar to N-9 but failed to affect the sperms in uterus cervical mucus even after prolonged contact. Saponins (12) extracted from Phytolacca dodecandra showed 3 times the activity of synthetic detergent available. 

Saponins of the Yugoslav plants Primula Volgwis, Gypsophila Paniculata and Cyclamen Persicum immobilized human sperm in vitro at 1:1000 dilutions. The spermicidal activity of fish oil polyunsaturated fatty acids and their sodium salts was comparable to that of propranolol. A pharmaceutical composition of lauryl betaine, lauramine oxide and monohydrated citric acid showed good spermicidal activity along with treatment of viral infections. An antifungal saponin Mollugogenol-A (13) isolated from the tropical herb Mollugo PentaPhylla showed spermicidal activity in vitro.
A volatile fraction of neem oil\textsuperscript{47} was reported to have spermicidal activity. Orcinol and Orsellinic esters\textsuperscript{48} were reported to be potent and safe spermicides. SM-3\textsuperscript{49} showed spermicidal activity similar to that of N-9 when tested along with it. Praneem a neem seed extract, reetha saponins and quinine hydrochloride showed potent spermicidal activity individually as well as in combination. The selected combination was formulated to show a dual benefit of potent contraceptive and an antimicrobial preparation.\textsuperscript{50} Sodium dodecyl sulfate\textsuperscript{51} (14) and related anionic surfactants were used for the prevention and control of pregnancy and sexually transmitted diseases.

\begin{center}
\includegraphics[width=0.5\textwidth]{14.png}
\end{center}

Two saponins and two flavonoids isolated from berries of \textit{Phytolaccadioica} \textit{L.} showed spermicidal activity.\textsuperscript{52} A new formulation of Vaginal Suppository called “Long Acting Sustained Release of Spermicide” (LASRS)\textsuperscript{53} has showed antimicrobial and spermicidal activities. Mono and di iodinated nonoxynol-9 derivatives were prepared and tested for spermicidal activity.\textsuperscript{54} Vanadocene dithiocarbamates\textsuperscript{55} (VDDTC, 15) has the most potent and stable spermicidal activity and may have clinical utility as an active ingredient of non-detergent type, safe, vaginal spermicidal contraceptives.

\begin{center}
\includegraphics[width=0.5\textwidth]{15.png}
\end{center}

GM-144,\textsuperscript{56} a novel lipophillic vaginal contraceptive gel-microemulsion was formulated. Di- and tri-hydroxylated cationic surfactants show promise as inexpensive topical contraceptive microbicides.\textsuperscript{57}
5. **Acrosin Inhibitors:** The most thoroughly studied acrosin inhibitors include 4-guanidinobenzoates\(^{58}\) and gossypol\(^{59,60}\) (16). 2’-carbomethoxy-4-guanidino benzoate has been found to be most effective. Trihydroxy naphthalene\(^{61}\) (17) derivatives and its analogs were synthesized and showed activity similar to that of gossypol.

![Gossypol](image)

Cysteamine, Cystamine, Phosphocysteamine showed spermicidal activity along with Acrosin inhibitory activity.\(^{62}\) The non-immunogenic peptides derived from Zona Pellucida glycoproteins showed spermicidal activity in vitro without inducing acrosome reaction in capacitated sperm.\(^{63}\)

6. **Natural Products:** Several natural products have been evaluated for their spermicidal efficacy. These include polyphenolic compounds from plant,\(^{64}\) alkaloids,\(^{65}\) magainins.\(^{66}\) Allitridum\(^{67}\) an active principle of garlic showed complete immobilization of human spermatozoa at the 7.5mg/mL dose in vitro. Nepalins 1,2 and 3 triglycerides\(^{68}\) from *Hedera nepalensis K.koch* showed spermicidal activity at 0.5, 0.25 and 0.125% respectively. Three new alkaloids were isolated from a Chinese plant *Melodinus Fusiformis* in which two showed significant spermaticidal and antitumor activity.\(^{69}\) Salannin\(^{70}\) (18), a limonoid bitter principle of the seed oil of *Azadirachta indica*, showed antiulcer, antibacterial and spermicidal activities.
In vitro study of pongamia seed oil from *Pongamia glabra* showed strong spermicidal activity.\(^7\) Curcumin, a plant-derived diferuloylmethane\(^7\) (19) compound has selective sperm immobilizing effect in addition to a previously studied anti-HIV activity. This compound may have potential clinical application as a novel intravaginal spermicidal agent for contraception and HIV prevention.

Various forms of sophorolipids\(^7\) (20) produced by *Candida bombicola* showed spermicidal and virucidal activities.

**7. Miscellaneous compounds:** Several other synthetic compounds that have been evaluated for their spermicidal efficacy are substituted imidazoles,\(^4\) zinc acetate,
acrylophenones,\textsuperscript{75} benzophenones,\textsuperscript{76} parabens,\textsuperscript{77} mixture of betaine and amine oxides,\textsuperscript{78} urea,\textsuperscript{79} hydrogen peroxide, mandelic acid, citric acid, malonic acid, maleic acid, caffeic acid,\textsuperscript{80} chlorpromazine and phenoxybenzamine. New structural steroid type ring systems of benzthiadiazolones\textsuperscript{81} (21) showed good spermicidal activities.

New indolyl phthalazinones and phthalazine\textsuperscript{82} derivatives showed marked spermicidal activity, which was ten times that of N-9. 1,4-naphthaquinone,\textsuperscript{83} which was capable of generating reactive oxygen species (ROS) showed spermicidal and antimicrobial activities. Pyrimidine-based nucleosides (22), AZT\textsuperscript{84} derivatives were synthesized as spermicidal contraceptives and virucides.

Promethazine hydrochloride\textsuperscript{85} (23) showed spermicidal activity at 0.4mg/mL concentration, which is more effective than Propranolol on human sperm.
Phenethyl-5-bromopyridyl thiourea (PDT, 24) and Dihydro Alkoxy Benzyl Oxo pyrimidine\textsuperscript{86} (DABO, 25) derivatives exhibited spermicidal activity as well as antiviral activity. A contraceptive ointment is manufactured from iodine complex and Wanfujin.\textsuperscript{87} Compositions and methods for trapping and inactivating pathogenic microbes and spermatozoa were studied.\textsuperscript{88} The Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger and Ca\textsuperscript{2+}-ATPase pumps reported to be present on the sperm membrane are responsible for maintaining the intracellular Ca\textsuperscript{2+} concentration that is involved in regulation of sperm function. 2’, 4’-dichlorobenzamil hydrochloride\textsuperscript{89} (benzamil), a Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange inhibitor showed spermicidal activity.

![Chemical Structure](image)

N-(4-nitro phenyl)-5-chloro salicylamide\textsuperscript{90} (27) was used as spermicide for replacement of N-9 in external-use contraceptive. AZT\textsuperscript{91} derivatives showed spermicidal and anti-viral activity. Octyl-D-glucopyranoside\textsuperscript{92} (OGP) can immobilize and kill the sperms depending on concentration. Cyclohexenyl thiourea\textsuperscript{93} (CHET, 28) non-nucleoside inhibitors (NNIs) of HIV-1 reverse transcriptase (RT) derivatives showed clinical potential as anti HIV spermicides.

![Chemical Structure](image)

Activated Carbon, as well as compositions and kits comprising the same, are effective for preventing pregnancy and sexually transmitted diseases including HIV.\textsuperscript{94} Metronidazole was formulated in a concentration of 5% as an acid gel, which showed instant immobilization, and
death of all sperms within 30sec. WHI-05\textsuperscript{96} (5-bromo-6-methoxy-5,6-dihydro-3’-azidothymidine-5’-(p-methoxyphenyl) methoxy alaninyl phosphate, is a highly promising antiviral and spermicidal agent. N-[2-(1-cyclohexenyl)ethyl]-N’-[2-(5-bromopyridyl)]-thio-urea\textsuperscript{97} (PHI-346) was studied as novel broad spectrum anti-HIV and spermicidal agent. OxoVanadium (V) complexes\textsuperscript{98} (29) with thiourea non-nucleoside inhibitors of HIV-1 reverse transcriptase showed potent dual anti-HIV and spermicidal activities.

Formulations containing at least one 5’-alkyl resorcinol and Cannabinoids or both were useful as barrier contraceptives.\textsuperscript{99} Niphensamide\textsuperscript{100} is hopeful to become a safe, highly effective, inexpensive, convenient and new contraceptive with anti-infectious activity. Hexahydro indenopyridines\textsuperscript{101} (30) were synthesized as spermicides and antifungals.

4[3(dialkylamino/heterocyclicamino)2methylene1oxoprop1yl]1O[3(heterocyclicamino/dialkylamino)-2-hydroxypropyl]thymols\textsuperscript{102} (31) and their salts were prepared as potential vaginal contraceptive agents.
Calcium hydroxide, Magnesium hydroxide and Aluminum hydroxide were formulated into gel form and used as contraceptive and antiviral powder.\textsuperscript{103}

Based on the above classification of the agents and keeping in mind the structural requirements for dual function so many numbers of new compounds were designed and synthesized for spermicidal activity against human sperm. On the basis of sub-structural analysis of gossypol, a polyphenolic spermicidal compound, a number of Mannich bases of substituted phenols\textsuperscript{104} were synthesized. Spermicidal activity was found in prototypes 32 \& 33\textsuperscript{105} 1,4-Bis (hydroxyphenyl) methyl piperazine (34), aryloxy propanol amines (35) and the cyclic analog of 31 namely the substituted 1,3-benzoaxazine (36) also elicited spermicidal activity.\textsuperscript{106}
Significant spermicidal activity was encountered in Michael acceptors such as  N-substituted α-aminoalkyl acrylophenones\textsuperscript{107} (37). Other compounds representing structure variants of compound 37 showed significant activity at 0.05, 0.1 and 0.01% concentration respectively. Other Michael acceptors possessing a quinoline ring, namely compound 38 (R=6-OMe, R\textsubscript{1} = C\textsubscript{3}H\textsubscript{7}; R=6-OMe, R\textsubscript{1} = (CH\textsubscript{2})\textsubscript{3}N(CH\textsubscript{3})\textsubscript{2}) were found to be active at 0.05% concentration.

Another structurally modified derivative of compound 38, namely the compound 39 was active at 0.02% concentration.\textsuperscript{108} However, large number of Michael acceptor compounds caused vaginal irritation and were not pursued further.\textsuperscript{109} This led to the synthesis of a number of acyclic Mannich bases, which were either poor acceptors or were incapable of undergoing 1,4-addition reactions. The active prototypes were \(\omega\)-amino propiophenones\textsuperscript{110} (40), 3-N-substituted 2-methylene indan-1-ones\textsuperscript{110} (41) and substituted styryl amino ketones\textsuperscript{111} (42).
Mannich bases derived from cyclised structures such as indanones (43, n=1), tetralones (43, n=2), and 6-keto estradiol (45) were also synthesized. Of these indanone (43: R=piperazine, n=1) and the estradiol derivative (45: R=pyrrolidine) showed MEC of 0.1 and 0.5 respectively. Spermicidal activity of the compound was lost when the carbonyl group was reduced (44). The oxime derivatives of tetralone 46 were inactive as spermicides.

The oxime derivatives of tetralone 44 were inactive as spermicides. The next class of spermicidal compounds was the acetophenone derivatives (47: R= CH$_2$-piperidine, R$_1$=piperidine), which exhibited activity at 0.01% concentration. Lower order of activity was observed in elaborated acetophenone of the type 48. (R$_1$ or R$_2$ is a substituted amine).
Quinone derivatives (49), were also investigated and most active compound was 50 (R=COCH₃) active at 0.05% MEC.

![Chemical Structure 49](image)

Thirty-one derivatives of benzophenones and naphthophenones (50 & 51) bearing a basic side chain were synthesized as spermicidal agents. Out of these, 50 (R₁=3-Me, R=CH₂CH₂N(C₂H₅)₂, R₂=H; R₁=4-OCH₃, R=CH₂CH₂NC₄H₉, R₂=4-OCH₃) and 51 (R=CH₂CH₂N⁺C₄H₉.CH₃I) were active upto 1% concentration. The biphenyl compound (50: R₁=4-Ph, R= R₂=H) was active at 0.05%.

![Chemical Structure 50](image)

![Chemical Structure 51](image)

Interestingly, reduction of the carbonyl function to CH₂, in bezophenone, prototype 52 gave the diphenyl methane derivative (52: R=4-OH, R₁=4'-OMe, R₂=2'-OH), which was active at 0.01% concentration. Some of the diaryl amino methane derivatives 53 also showed promising activity. Most active compound of this series was amino ethanol 54 (R₁=5-Me, R₂=2-OH, R₃=H, R=CH₂CH₂OH) active at 0.5% concentration.
The compound 54 and derivatives were investigated as spermicidal agents. Compound 54 was found active at 0.05% concentration. Substituted β-phenylethyl amines with a residue on the α-carbon were synthesized as spermicides. Compound containing a cycloheptane ring were found to have better spermicidal activity. Most active compound of the series was 55 (R=2-Cl, n=2) active at 0.01% conc. followed by 55 (R=4-CH₃, n=2) active at 0.05% conc.

The reactive oxygen and nitrogen species are powerful initiators of lipid peroxidation, thereby are likely to induce changes in the membrane fluidity. Compounds capable of generating radicals may mimic the reactions of ROS and finally lead to the damage of spermatozoa. Nitro compounds are well known for their capacity to generate free radicals. On this basis structural types 56-59 were synthesized. Some of the nitro compounds of type 57 (R=H, n=1, MEC 0.5) and 58 (R=4-CH₃, R₁=H, n=2, MEC 1.0; R=4-OCH₃, R₁=CH₂CH₂CN, n=2, MEC 1.0) showed activity.
Attempt was made to design compounds that would disturb the equilibrium between the amount of ROS produced and that scavenged by a cell by interfering with enzymes involved with the latter. Thus, a number of isoxazoles\textsuperscript{115} \textbf{60, 61} were synthesized. The most active compound of this series was active at MEC of 0.005\%. This compound also exhibited anti-HIV activity in vitro assay.

Compound prototypes \textbf{62-64}\textsuperscript{120} were synthesized to interact with sperm membrane either directly or through oxidoreductase process of the enzyme present in seminal plasma. However, these compounds were less active.
Maleimides represent a class of biologically active compounds possessing potent microbicidal, fungicidal, anti-enzymatic and various other activities. Compounds of prototype 65 were therefore, synthesized to evaluate their possible spermicidal activity.

The most active compound of this series is 65\(^{121}\) (R=H, R\(_1\)=OH, Ar=C\(_6\)H\(_5\), MEC 0.5%).

No significant activity was observed in triazoles (66, 67) and triazolo pyrimidines\(^{122}\) (68).

However, the cyclohexyl pyrazoline (69) substituted with a basic chain showed MEC of 0.5%.
2-Nitrobenzylidine-1-cycloalkanones (70), 2,6-bis-benzylidene derivatives (71), and 2-nitrobenzylidine-1-indanones (72) were synthesized as spermicidal agents. The most active compound of the series was 70 (n=2, p-NO₂).

![Chemical structures]

Significant spermicidal activity was observed in some of the compounds belonging to the polymethylene class of amines 73, amino alcohols 74 and butyrolactone derivatives 75.

![Chemical structures]

Thiadiazolo [3,2-a] pyrimidine derivative 76 showed MEC of 0.05%.
Sodium salt of dithiocarbamic acids 77, alkyl/cycloalkyl/heteroalkyl esters of dithiocarbamic acids (78) were prepared as spermicidal agents.\textsuperscript{124, 125}

![Chemical structure](image)

\( R_1 = \text{Et or morpholine}, \text{MEC} 0.5\% \) and \( R_1 = \text{Et}, R = \text{CH}_2\text{COOH MEC} 0.1\% \) possessed spermicidal activity.

Compound 77 \((R_1=\text{Et or morpholine}, \text{MEC} 0.5\% \) and \( R_1=\text{Et}, R=\text{CH}_2\text{COOH MEC} 0.1\% \) possessed spermicidal activity.

Compounds of the structural type 1-aryl or alkyl-4-substituted aminomethyl penta-1,4-dien-3-ones were prepared as spermicidal agents.\textsuperscript{126, 127}

Substituted acrylophenones\textsuperscript{128} (79) and related Mannich bases were synthesized as possible spermicides and inhibitors of HIV envelope glycoprotein-CD4 interaction.

![Chemical structure](image)

Substituted Quinotoxine derivatives\textsuperscript{129} were synthesized to prepare water-soluble vaginal contraceptive.
In plant products, a number of modified analogues of loganin\(^{130}\) (80) and solanesole derivatives (81) were submitted for spermicidal activity evaluation. None of the compounds showed any significant activity.

![Diagram of compound 80]

The synthesized marine product and its derivatives having a lipophillic chain 82 showed activity at 0.5% concentration and also possessed anti-HIV activity.

![Diagram of compound 82]

Certain sugar derivatives (83), particularly glycofuranones having amino acid as appendages have been reported to possess acrosin inhibitory activity and they also show antiviral property. Based on this finding 3-O-aminoalkyl ethers \(\alpha\)-glucofuranone were synthesized as spermicidal agents. Compounds belonging to prototype 80 showed MEC of 0.5-1.0%.
The spermicides in current use:

The great majority of vaginal contraceptive preparations that are available throughout the world rely on surfactants as the spermicidal agent. The most commonly used compound is the neutral surfactant nonoxynol-9. Other spermicidal products use either the structurally related compound octoxynol, or an alternative type of non-ionic surfactant, p-di-isobutyl-phenoxypolyethoxyethanol. Worldwide, the cationic surfactant benzalkonium chloride, the anionic detergent sodium docusate (dioctyl sodium sulfosuccinate, 84), and the neutral surfactant Menfegol (p-menthanylphenyl polyoxyethylene, 84) are also used.

Surfactants probably have a common mode of spermicidal action, though most of the research in this area is concentrated on nonoxynol-9. This compound is spermicidal by virtue of its detergent action on the lipid components of the membranes of the mid-piece and tail regions of the spermatozoa, resulting in the rapid disruption of the membranes and loss of motility. This mechanism is not selective and any unprotected lipoprotein membrane will be susceptible to disruption, including those of vaginal organisms. In addition to the direct detergent action, a
cationic structure could have enhanced bactericidal lysis as the surfactant would be attracted or bound to the acidic components that are present in, or associated with, the bacterial wall, e.g. sialic or teichoic acid. Furthermore, it was proposed\textsuperscript{132} that non-ionic surfactants that have ether or amide linkages between the hydrophobic and hydrophilic domains of the molecule could have a virucidal action by virtue of their ability to dissolve the lipid components of the viral envelope. This prediction was made after the observed destruction of the envelope of herpes virus after in vitro exposure to nonoxynol-9. Several authors have suggested that nonoxynol-9 may have role to play in the regulation of transmission of herpes virus during intercourse.\textsuperscript{133,134} This potential dual action towards both spermatozoa and microorganisms forms the basis of the proposal that spermicides can have a role in the prevention of infection by STDs. There is, however, no evidence to support a therapeutic role towards pre-existing infections.

Evidence for the restriction of the spread of STDs by spermicides was reported\textsuperscript{135} in 1972, when it was shown that \textit{Treponema pallidum}, \textit{Neisseria gonorrhoea}, \textit{Trichomonas vaginalis} and \textit{Candida albicans} were inactivated by exposure in vitro to the levels of non-ionic surfactant spermicides that could be expected to be present in the vagina during intercourse when a spermicide was used. This has been supported by other studies of women at high risk of STD infection, when the rate of reinfection was drastically reduced if a spermicide was used.\textsuperscript{136,137} However, spermicides will not cure pre-existing infections. Subsequently it has been shown that nonoxynol-9 has activity against a wide range of bacterial, and parasite-associated STDs; activity against viral infections has been demonstrated only in vitro.\textsuperscript{138} Currently there has been a resurgence of interest in the action of spermicides against STDs in relation to the spread of HIV in the heterosexual population. It was first shown in 1985 that nonoxynol-9 will inactivate both cell-based HIV and the free virus in vitro, at concentrations well below those expected to be present in the vagina when a spermicide is used.\textsuperscript{139} It is important to note that no data exist to show that spermicides are active against HIV in vivo.\textsuperscript{140}

There are several factors that must be considered when assessing the true value of spermicides in the limitation of infection with STDs in the heterosexual population.

1. Much of the evidence for the use of spermicides against STDs relates to their antiseptic action in vitro. This approach does not consider the possible attenuation of the antiseptic
activity by its dilution by the male and female secretions, its speed of action, binding of
the spermicide to proteins, or possible pH dependence of its action under the conditions
that exist in the vagina during intercourse. One of the major considerations in this area is
the lack of entry of nonoxynol-9 into cervical mucus at the concentrations that would be
present vaginally after the use of a spermicide.\textsuperscript{141, 142} This property limits the extent to
which this compound is active in the genital tract. Additionally, specific STDs have a
preference for different parts of the genital tract, for example gonorrhea mainly infects
the cervix and endometrial tissue, and therefore the extent of penetration of a spermicide
into the genital tract is an important consideration in the prevention of infection.

2. Evidence exists showing that high levels of nonoxynol-9 (above those that would occur
after a single application of spermicide) cause lesions and severe ulceration of the vaginal
epithelium of laboratory animals.\textsuperscript{143, 144} The adverse vaginal reaction to spermicides
appears to be dose related, the usual rate of use being about 100mg surfactant at coitus.
This level is, however, about 30 times greater than the ED\textsubscript{100} (the lowest concentration of
nonoxynol-9 in semen in vitro, that results in 100\% loss of motility 1min after contact),
which has been shown to be in the region of 0.3mg/ml.\textsuperscript{145} It could also be predicted that
damage to the epithelium would enhance the entry of STDs. Furthermore, damage to the
epithelium of this sort could lead to increased numbers of T-lymphocytes and
macrophages in the vagina which could increase the risk of infection by HIV. Indeed
there is some evidence to support this suggestion. In a study conducted amongst
prostitutes in Nairobi, in which some of the women used nonoxynol-9-containing
sponges, a significantly higher rate of genital ulceration and HIV seroconversion was
found with those not using nonoxynol-9\textsuperscript{146} and it is possible that the abrasive action of
the sponge was an important factor in these findings.

3. The final qualifying factor in the use of spermicides as prophylaxis for STD is the effect
of these compounds on the normal microbial species in the vagina. As spermicides have a
degree of bactericidal action it is pertinent to question the effect of their frequent use on
the vaginal flora with associated repercussions upon the vaginal pH, possibly leading to
an overgrowth by pathogenic species (that could be sexually transmitted). There is a
reported spectrum of sensitivity of the vaginal flora to nonoxynol-9; many of the species of Lactobacilli that are found in the vagina are susceptible to nonoxynol-9 after extended contact time in vitro.\textsuperscript{147}

Though nonoxynol-9 was the widely used surfactant is a detergent that disrupts cell wall by solubilizing membranes, have several disadvantages. N-9 increases the risk of urinary tract infections,\textsuperscript{148, 149} vulvovaginal candidiasis,\textsuperscript{150} and genital ulcers.\textsuperscript{151} As human trials show, N-9 may increase the risk of HIV transmission,\textsuperscript{152} perhaps by initiating interleukin-1-mediated NF-kB activation, which leads to cytokine-induced recruitment of HIV-1 host cells and increased HIV-1 replication.\textsuperscript{153} N-9, a mixture of oligomers,\textsuperscript{154-158} may violate future federal regulations; pure compounds or mixtures whose individual components have met safety standards will become the norm. Furthermore, the breakdown products of N-9 pose serious health and environmental risks.\textsuperscript{159} The trafficking HIV-infected mononuclear cells in semen are the likely source for sexual transmission of HIV, the infected seminal cells could easily come in direct contact with target cells in the mucosa of sexual partners after N-9 induced disruption of cervicovaginal epithelial barrier, resulting in viral transmission. Therefore, dual function microbicides lacking detergent type membrane toxicity would have advantage over the currently used ones.

Thus none of the agents, synthetic or natural, known so far having spermicidal activity fulfill the essential requirement of a modern 21st century spermicide i.e., having action against both the spermatozoa and the organisms causing STD and AIDS without effecting the vaginal flora. The requirement for a dual-function spermicide can be delineated as below:

- Potent spermicidal efficacy
- Efficacy against HIV infection
- Efficacy as antibacterial and antifungal agent
- Minimal effect on vaginal flora
- Minimal membrane toxicity
- Economic feasibility

To synthesize a dual function spermicide with above specialties there is need to know the biology of sperm.
**Structure of sperm and its production:**

Sperm is produced in the male’s testis, which is composed of about 200 meters long highly convoluted seminiferous tubules having two specialized cells

2. Sertoli cells and
3. Epithelial lining.

Proliferation of stem cells in the epithelial linings leads to the production of innumerable spermatozoa through a process called spermatogenesis. Sertoli cells (picture 1) provide nutrition and support that are requirements of spermatogenesis. The spermatozoa that are released into the lumen of the seminiferous tubules are immobile and are incapable of fertilization. The spermatozoa reaching the epididymis undergo further maturation and acquire the fertilization capacity and the potential for progression motility.

An agent, which will interfere with the production and maturation of the spermatozoa, is called an antispermatogenic agent and is male fertility regulatory agent.

Seminal fluid containing large number matured spermatozoa (about a hundred and thirteen million per ml) when released in the vaginal cavity passes through the cervix and through uterus. The sperm has a sperm head and a tail as shown in the picture –1.

The outer glycocalyx represents charged glycolipid and glycoprotein residues with oligosaccharide chains. The membrane matrix is made of protein in which the lipid bilayer with hydrophilic chain directed outwards and hydrophobic end inwards. The submembrane is composed of cytofibrilar system of microtubules and microfilaments, which are precisely cytoskeletal and contractile elements. The major components of glycocalyx, glycolipids and glycoproteins, acts as biosensor for ligand such as hormones, drugs, antibodies and toxins. Glycosyl transferases and glycosides help the assembly of carbohydrate moieties. The sperm head carries acrosomal enzymes and the tail enables the sperm to move. By this tail movement the sperm moves up the oviduct.
The ovum released from the ovary in the female is immobile and is sucked in from the other end of the fallopian tube by the movements of ampulla. Ova thus released have a protective multiple layer coating on it. Interaction of the sperm with ova gradually results in the dissolution of the layering by the proteolytic enzyme released from the sperm head. One of the sperms ultimately finds its way to the ova through the cavity made in its protective lining and results in fertilization. In the above process immobilization of the sperm after its release in the vaginal cavity would result in the interference of the process of fertilization. An agent, which causes immobilization of the sperm, is called a spermicidal agent. Since the process of inactivation of sperm mobility is achieved in the female, these agents are grouped under female fertility regulating agents.

Spermicides exert an antifertility effect upon spermatozoa as it passes through the female genital tract. To be an effective spermicidal agent, a compound must meet the following requirements. It must act rapidly and efficiently to kill or immobilize sperm on contact, or render the sperm incapable of fertilization. It must be nonirritating to the vaginal and penile mucosa, should not have any adverse effect on the developing embryo or fetus, and must be free of long term toxicity. Moreover, it should be systemically nontoxic.
Spermatozoa must undergo capacitation prior to fertilization. In humans, this process appears regulated by oxidoreduction reactions. Sperm capacitation is associated with a low production of reactive oxygen species\textsuperscript{160-162} (ROS) and a strong time-dependent increase in sperm membrane sulfhydryl groups\textsuperscript{163} Sulfhydryl groups of sperm membrane proteins maintain a dynamic equilibrium with their disulfides counterparts.\textsuperscript{164} Oxidation of sulfhydryl groups is associated with the acquisition of fertilizing ability of spermatozoa.\textsuperscript{165} In rat epididymis, caput ligation caused an oxidation of sperm sulfhydryl groups triggering an early maturation of the gametes as evidenced by the acquisition of motility and of a partial increase in fertilizing ability.\textsuperscript{166} 

Sulfhydryl groups of the sperm membrane play a very important role in sperm motility and metabolism\textsuperscript{167} and evidence is also available for the involvement of these surface thiols in normal sperm functioning.\textsuperscript{168} The loss of sperm surface thiol groups and the augmented production of superoxide anion radical was stated to be the reason for the loss of motility.\textsuperscript{169} The sulfhydryl blocking properties of both copper and cobalt is utilized to study role of sulfhydryl groups in sperm membrane modulation. Reports show, a possible regulatory role of sulfhydryl groups in sperm membrane modulation and as a marker for fertility assessment.\textsuperscript{170} 

Membrane integrity and its proper functioning are the basic characteristics of the sperm membrane including cellular recognition and information transduction during cell cell interaction. Changes in fatty acid composition of membranes as well as the amount of individual sterols, account for the change in fluidity. Normal spermatozoa are reported to show high membrane fluidity.\textsuperscript{171} Therefore it is evident that membrane fluidity is an important factor for sperm functions. Lipid peroxidation plays a crucial role in inducing membrane fluidity.\textsuperscript{172} The results presented showed that blocking of sperm membrane sulfhydryl groups both in human and rat caudal sperm inhibits lipid peroxidation and superoxide dismutase activity.\textsuperscript{170} 

It has been reported earlier that copper containing antifertility devices are believed to act via production of toxic oxygen radical species including hydroxyl and superoxide anion radical\textsuperscript{173} and these radical species are known to induce membrane fluidity via lipid peroxidation. But, contrary to the above statement it was found that blocking of sulfhydryl groups by copper and cobalt inhibits lipid peroxidation in normal spermatozoa and treatment of oligospermic samples with pentoxifylline increases lipid peroxidation.\textsuperscript{170} However, this is a unique phenomenon, which remains to be elucidated. But it is quite evident that membrane sulfhydryl groups play an
important role in sperm membrane modulation by reducing membrane fluidity. Decrease in motility and loss of sperm function in unexplained male infertility can be attributed to these sulfhydryl groups of the sperm membrane and is suggested that in such cases of infertility the active sulfhydryl groups are masked which results in loss of sperm. Thus, it is suggested that sperm membrane sulfhydryl groups are important entities of the membrane and can be used as a tool for infertility assessment in unexplained male infertility and can be targeted for contraceptive research.\(^{170}\)

According to the various conditions (medium, stimulant, etc.) used by different researchers, \(\text{O}_2\cdot\text{O}_2\), \(\text{H}_2\text{O}_2\), or both of these oxidants induce human sperm capacitation\(^{174-178}\). These observations converge to emphasize again the concept that sperm capacitation is part of an oxidative process. However, the extracellular sperm membrane target for \(\text{O}_2\cdot\text{O}_2\) during capacitation remains elusive. Characterized by short half-life (1 ms) and a low reactivity, \(\text{O}_2\cdot\text{O}_2\) is usually not considered as a potent lipid peroxidation inducer. However, \(\text{O}_2\cdot\text{O}_2\) can react with sulfhydryl groups (-SH) then forming thyl radicals (2S\(^+\)), which can subsequently create a disulfide bridge (-SS-) or a thiolation product with other sulfhydryl containing substances such as glutathione\(^{179}\). The sulfhydryl-disulfide status appears important in epididymal sperm maturation but little is known on its role in capacitation. The results presented strongly suggest that the sulfhydryl-disulfide pair is involved in the regulation of human sperm capacitation and the associated \(\text{O}_2\cdot\text{O}_2\) generation. The reversibility of oxidoreduction reactions involving sulfhydryl and disulfide groups and the possible shuttle between these two forms are compatible with the reversibility of the capacitation process.\(^{178, 180}\) In addition, the fine control of the sulfhydryl-disulfide pair could offer a mechanism by which sperm capacitation would be prevented until appropriate conditions (location in the female genital tract, timing, etc.) occur and then be induced. However, the factors involved in this fine control of the sulfhydryl-disulfide pair appear complex and multifactorial and may involve many parallel or associated signal transduction pathways leading to sperm capacitation.

As it is suggested that sperm membrane sulfhydryl groups are important entities of the membrane and can be used as a tool for infertility assessment in unexplained male infertility and can be targeted for contraceptive research, we wanted to explore the sulfhydryl-binding agents. From the literature survey, it is evident that very limited work was done on sulfhydryl-binding
agents. Hydrogen peroxide, o-iodobenzoate, several hydroquinones, phenyl mercuric acid, Maleimide derivatives like compounds are reported for their spermicidal activity and all compounds show the thiol-disulfide conversion in mode of action.

N-Ethylmaleimide, a sulfhydryl-selective alkylating agent and its derivatives have been found to possess spermicidal activities, which is attributed to their interaction with the sulfhydryl groups present over sperm cell membrane. Since binding to sulfhydryl groups of sperm membrane is important for the spermicidal activity, and paroxetine. HCl, a SSRI antidepressant, is known to bind serotonin transporters by interacting with sulfhydryl groups, it was suspected that SSRIs could possess spermicidal activity. Further, Spermicidal action of these SSRIs might be attributed to their effect on ATP synthesis by inhibition of oxidative phosphorylation in sperm mitochondria as exhibited by Fluoxetine.HCl in rat liver and brain mitochondria. This inhibition by Fluoxetine.HCl was found to be non-specific and indirect mediated via its interaction with phospholipids in the inner mitochondrial membrane. The spermicidal activity of these SSRI antidepressants may also arise due to their possible interaction with sulfhydryl groups present over sperm membrane. This might be considered as their non-detergent mode of action.

Tricyclic antidepressants, Amytriptilin and Imipramine have been shown to possess sperm immobilizing activity and certain SSRI antidepressants have been found to possess antifungal activity. Additionally, serotonin functions as a neurotransmitter in brain, as well as in a number of other tissues including testis, where it exerts effect by binding to cell surface receptors.

*Trichomonas vaginalis*, the most common, non-viral STD, infects 250 to 350 million people worldwide every year causing serious discomfort to women with associated problems of adverse pregnancy outcome, pre-term delivery, low-birth-weight infants, infertility and cervical cancer besides also an increase in the transmission of HIV. These three SSRI antidepressants viz. Fluoxetine.HCl, Sertraline.HCl and Fluvoxamine maleate have shown remarkable antitrichomonas activity comparable to N-9, the commercially available spermicide. This activity might be explained according to the finding that SSRIs primarily act at the serotonin transporter protein (SERT) and block the reuptake process of serotonin. SERT, with molecular weight of 60-80 kDa and 12 transmembrane domains, is similar to other biogenic amine
transporters.\textsuperscript{185} It may be presumed that their antitrichomonas activity might be resulting from an interaction of SSRIs and membrane transport system, as has been reported for \textit{Staphylococcus aureus} and chlorpromazine.\textsuperscript{189} Thus SSRI antidepressants exhibiting both the spermicidal and antitrichomonas activity may provide a lead structure for the development of novel, non-detergent, dual-function microbicidal-spermicides. Of particular interest is fluoxetine with a noticeable spermicidal and microbicidal activity. Such molecules can be developed as suitable alternatives to N-9.

These observations prompted us to evaluate the spermicidal activity of N-alkylmaleimide derivative and also phenylpropanamine analog included in the study to compare its effect on sperm viability. Since spermatozoa and several of STD-causing microbes share common mechanisms of action,\textsuperscript{187} it was considered worthwhile to test their spermicidal as well as anti-STI activity against \textit{Trichomonas vaginalis}. N-9 was used as reference standard in this study.

Isoindole-1,3-dione and Fluoxetin was taken as the lead structure for optimization of spermicidal activity. In order to optimize the activity following modifications were made in the corresponding structure:

1. Dithiocarbamate derivatives of Isoindole-1,3-dione
2. Derivatisation of fluoxetine structure

1. Dithiocarbamate derivatives of Isoindole-1,3-dione
   1.1 dialkylamino -1-carbodithioic acid 3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-2-hydroxy-propyl ester

\[
\text{NR}^1\text{R}^2 = \text{Substituted aminoalkyl}
\]
2. Derivatisation of Fluoxetine structure

1-(Substituted)-3-(4-(substituted) amino)-phenylpropanamines

2.1 3-(substituted amino-1-yl)-1-phenylpropan-1-one

\[
\begin{align*}
X &= CO, \\
NR^1R^2 &= \text{Substituted amino}
\end{align*}
\]

2.2 Synthesis of 3-(substituted amino-1-yl)-1-phenylpropan-1-ol

\[
\begin{align*}
X &= OH, \\
NR^1R^2 &= \text{Substituted amino}
\end{align*}
\]

2.3 Acyl protected-3-(substituted amino-1-yl)-1-phenylpropan-1-ol

\[
\begin{align*}
NR^1R^2: &-N\ce{O} -N &-N\ce{N}C_2H_5 &-N\ce{N} &-N\ce{N} \N\ce{N} &-N\ce{N} \N\ce{N} &-N\ce{N} \\
&-N\ce{N} &-N\ce{N} &-N\ce{N} &-N\ce{N} &-N\ce{N} &-N\ce{N} &-N\ce{N} \\
\end{align*}
\]
3-substituted-1-phenyl-1-(propoxymethyl)-mono/dialkyl-amine

NR\textsuperscript{1}R\textsuperscript{2}: \text{N} \text{N} - \text{C}_{2}\text{H}_{5} - \text{N} \text{N} - \text{N} - \text{N} - \text{N} - \text{N} - \text{N} - \text{N} - \text{N} - \text{O} - \text{N} - \text{C}_{2}\text{H}_{5}