Review

Spermicidal agents

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ABSTRACT: In recent years, there is a development of vaginal contraceptives incorporating potent spermicides. Many compounds with different pharmacological activity have been evaluated *in vitro* for their spermicidal activity. Drugs such as surface-active agents (synthetic and natural), ionophores, antiliquefying agents, antimicrobial agents and miscellaneous agents such as gossypol, *Azadirachta indica*, vanadocenes have all been demonstrated to possess good spermicidal activity. Nonoxynol is the only spermicidal agent currently marketed and widely used. But there is still a need to develop alternative compounds for future use as safe spermicide.

Keywords: Spermicidal agents, Nonoxynol, Neem, Plants with spermicidal activity, Antifertility agents

1. Introduction

The world population continues to grow at an alarming rate, with a projected 50% increase in current world population to approximately 9 billion by 2050. Many methods such as condoms, oral contraceptives and intrauterine devices are available since long but there is still a quest for alternative means. A vaginal topical is the primary, if not the only, technique whereby, a women can prevent both pregnancy and infections. The serious development of chemical spermicides for public use dates back to the 1930's. Research has focused on the development of safe, highly effective and inexpensive spermicidal agents as one of the several alternative methods for family planning.

Spermicides are a biologically obvious way of interrupting fertility and have advantage that they do not depend on high skilled personnel for their prescription and use. Spermicidal agents are defined as drugs that have the ability to immobilize or kill the sperm upon contact. An ideal spermicide should immediately and irreversibly produce immobilization of the sperm, non-irritating to the vaginal and penile mucosa, not have adverse effects on the developing fetus, free from long-term topical and systemic toxicity and should not be systemically absorbed.

Hence, the spermicidal agents should be critically evaluated for these aspects. Understanding the morphology of spermatozoa is essential to appreciate the mechanism of action of spermicide.

2. Morphology of spermatozoa

Each normal spermatozoon is made up of two parts a head and a tail. The head consists of two main parts, the nucleus and acrosome. The nucleus contains the whole of the chromatin content of the sperm and the acrosome is made up of collection of enzymes that will aid penetration of the zona-pellucida by the sperm. The connecting piece is a small area in a very short segment that joins the head to the tail. The sperm tail is the means by which the sperm moves. The tail of the sperm consists of three parts the middle piece, the principle piece and the terminal segment. Overall the tail measures around 50 μm. The whole length of the tail contains a central contractile unit known as axoneme. In the middle piece, a central axoneme is present which is surrounded by closely packed helix of mitochondria. The principle piece makes up more than 90% of the length of the tail followed by the terminal segment. The sperm plasma membrane serves as a continuous limiting cell boundary, maintaining cell integrity and forming a dynamic interface between the cell boundary and its immediate environment (*1*,*2*).

One of the most challenging pursuits in the realm of pharmaceutical and medical sciences is the search for newer and more potent spermicides with little or no toxic effects and available at reasonable cost. A review of spermicidal agents which are in various stages of preclinical and/or clinical stage of development are given as follows:

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3. Spermicides acting through pH modification

The spermatozoa are motile between pH 6.7 to 8.5. Therefore, one of the oldest approaches for achieving spermicidal action has been to modify vaginal pH. The normal human vaginal pH is 3.8-4.2. This naturally acidic environment is maintained by the production of lactic acid by the vaginal flora. HIV, several STD-causing microbes and spermatozoa are inactivated at this low pH. When semen enters the vagina, the pH rises to above 6.0 because of the buffering activity of the ejaculate (pH 7.2-8.0) (*3*). It is well documented that sperm are sensitive to low pH and acidic solution can immobilize sperm within minutes (*4*).

ACIDFORM, an acid buffering vaginal formulation that maintains the acidic vaginal pH below 5.0 when ejaculate is deposited in the vagina or when a vaginal infection is present. In women, the desired acidification of semen can be achieved with a 3-5 mL dose because the average volume of the human ejaculate is about 3 mL, which would require less than 1 g of ACIDFORM to buffer the semen to a pH lower than 5.0. ACIDFORM is slightly off-white in appearance with a pH of about 3.55. The formulation consists of gelling agents, buffer salts, humectants, preservatives and water that are all GRAS except for one, which although not GRAS, is currently used in marketed vaginal formulations. A recently completed Phase I clinical safety study with ACIDFORM confirms its safety. No patient complaints (symptoms) have been recorded when ACIDFORM was applied vaginally for six consecutive days and no vaginal or cervical irritation was noted on visual or coloscopic inspection (*5-8*).

Lemon juice has been used as traditional intravaginal contraceptive throughout the Mediterranean region for hundreds of years. The spermicidal properties of lemon juice are possibly due to the high concentration of citric acid (*9*). Investigations have demonstrated that dyein ATPase in the sperm midpiece is required to energize the sperm tail. The acidic pH of lemon juice may immobilize sperm by denaturing dyein ATPase (*10*). Clarke *et al*. have demonstrated that a minimum concentration of 200 µL/mL lemon juice would be required to irreversibly immobilize 100% of spermatozoa. Ejaculate volume in normal men rarely exceeds 5 mL, so it would be necessary to deliver at least 1.5 mL of lemon juice into the vagina to obtain the desired concentration (*9*). Lemon juice would be a cheap and widely available vaginal contraceptive if its safety and efficacy were demonstrated (*11*).

4. Synthetic surface active agents

4.1. *Non-ionic surfactants*

In the category of non-ionic surfactants octoxynol and nonoxynol are the two commonly reported surfactants.

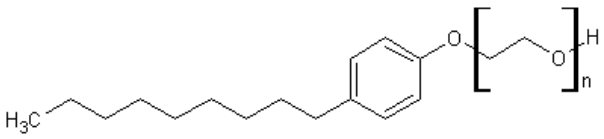

Figure 1. Structure of Nonoxynol-9.

Nonoxynol is more potent spermicide than octoxynol. Among the different nonoxynol derivatives designated as N1-N15, *p*-nonyl phenoxy polyethoxy ethanol (N-9) is reported to be the most potent spermicide (Figure 1) (*12*). At present N-9 is the only marketed contact spermicide available worldwide.

N-9 destroys the cell membrane of the neck of spermatozoa. The SEM/TEM of spermatozoa have revealed that after contact with N-9 the plasma membrane and the acrosomal membrane complex were removed, the midpiece membrane was absent, the normal cristae of the mitochondria were empty and the fibers were exposed. Damage to all membranes was first evident as vesiculations. Membranes then became loose and detached. The observed irreversible severe membrane alterations cause an immediate devitalization of the spermatozoa (*13,14*). At the dose of 50 µg/mL N-9 completely abolishes all sperm movement within one minute of addition. Although, N-9 has been employed as a contact spermicide for the last 30 years and is well tolerated, reports have appeared suggesting that the frequent use of surface active spermicides, can be associated with vaginal irritation and the appearance of lesions in the epithelium. The most common abnormality reported has been superficial de-epithelization of either the cervix or the vaginal walls, though there have been no reports of vaginal inflammation (*15,16*). In contrast, more recent studies reports that the use of N-9 as a microbicide or N-9 used in limiting the transmission of STD's should be discontinued since N-9 interferes with the lipid bilayer of the vaginal epithelium and facilitates the process of absorption and transmission of the viral particle into the blood stream (*17-20*).

Nonoxynol-9 is commercially available as gel, cream, foam and pessary formulations in various strengths (Table 1).

Ahmad N *et al*. have designed a new bioadhesive suppository of N-9 called Long Acting, Sustained Release of Spermicide (LASRS). The formulation is reported to adhere well to cellulose membranes *in vitro* and was completely spermicidal in the primate (stumptailed macaque) on post-coital tests even when mating was delayed for 12 h. Vaginal irritation studies in the rabbit and primate showed LASRS to be acceptable even with a nonoxynol-9 dose as high as 22.5% (w/w) (*21*). No coloscopic or visual vaginal lesions were induced when LASRS with 20% N-9 was used for 7 consecutive days by the volunteers participating in a pilot clinical trial. These studies

Table 1. List of marketed spermicidal formulations of nonoxynol

Sr. No.	Brand name	Nonoxynol-9	Formulations	Companies
1.	Advantage 24	3.5%	Gel	Columbia Laboratories
2.	Conceptrol Gel	4%	Gel	Advanced Care
3.	Koromex Crystal Clear gel	3%	Gel	Quality Health
4.	Ramses Personal Spermicidal Lubricant Gel	3%	Gel	London International U. S. Holdings
5.	VCF Gel	3%	Gel	Apothecus Pharmaceutical
6.	Encare	100 mg	Suppository	Thompson Medical Co.
7.	Semicid	100 mg	Suppository	Whitehall Robbins Healthcare
8	Koromex	125 mg	Suppository	Quality Health
9.	Gynol II	2%	Jelly	Advanced Care
10.	K-Y Plus	2.2%	Jelly	Johnson & Johnson
11.	Koromex	3%	Jelly	Quality Health
12.	Ortho- Gynol	1%	Jelly	Advanced Care
13.	Shur-Seal	2%	Jelly	Milex Products
14.	Delfen	12.5%	Foam	Advanced Care
15.	Emko	12%	Foam	Schering-Plough Healthcare
16.	Koromex	12.5%	Foam	Quality Health
17.	Ortho-Creme	2%	Cream	Advanced Care
18.	Today	5%	Pessary	Bliss Pharmaceuticals

suggest that LASRS possess advantages over presently marketed formulations by having long-term efficacy and by forming a bioadhesive, protective layer over the genital tract epithelium (*22*).

4.2. *Cationic surfactants*

Benzalkonium chloride is a bactericidal cationic surfactant, of the ammonium series that ceases the sperm flagellar motility immediately upon contact with spermatozoa. Four seconds after contact, the midpiece and head are destroyed. In concentrations of 70-300 µg/mL, the spermatozoon motility decreases, acrosomal proteins disappear, the fecundity capacity is lost as determined by hamster-ova penetration test and the enzymes of carbohydrate metabolism are disturbed. Benzalkonium chloride also coagulates ovulatory cervical mucus, its colloid network structure disappears and results in a magma with mesh of less than 5 µm, which is not permeable to spermatozoa. This action might be added mechanism of barrier to sperms in addition to its spermicidal activity. Vaginal suppository containing 18.9 mg of benzalkonium chloride have shown cervico-vaginal erosion/inflammation with the use of this suppository, which disappeared after cessation of use (*23*). It is interesting to note that concentration of benzalkonium chloride required for its spermicidal action is much less than that permitted for its preservative action (0.01-0.25%). Benzalkonium bromide in the concentration of 0.27 mg/mL also significantly affects the motility of human sperm and can be used as a spermicide (*24*,*25*).

Other cationic detergents like cetyl ammonium chloride and cetyl trimethyl ammonium bromide are also potent spermicides and instantly immobilize the spermatozoa at the concentration of 1 mg/mL. However, changes in the permeability of vaginal membrane on continuous use of these agents have been reported, and thus none of these agents are suitable for human use (*26*).

5. Natural surface-active agents: Saponins

Saponins are natural surfactants widely occurring in many plants and are reported to have spermicidal action. A common lipid bilayer, which contains external, internal and transmembrane proteins, is fundamental feature of the plasma membrane of the sperm. Saponin molecules interact with this lipid bilayer, affect the glycoproteins of the cellular membrane and modify the ionic transport across the membrane, leading to surface changes. These changes, namely vesiculation, vacuolation or dissolution of head region may occur due to stretching, loosening, breakdown of the membrane and ultimate removal of the acrosome (*27*).

Some of the saponin containing plants includes:

5.1. *Acacia auriculiformis*

Mixtures of two partially isolated triterpenoid saponins from the powdered seeds of *Acacia auriculiformis* (Acaciaside A and B) have shown spermicidal activity at the concentration of 0.35 mg/mL. The aglycone parts of these two saponins were characterized as acacic acid lactone and monosaccharide constituents were identified as D-glucose, D-xylose, L-arabinose and L-rhamnose. The complete chemical structures of these compounds are given in Figure 2. Electron microscopic observation showed that the plasma membrane was disintegrated and total dissolution of the acrosomal cap was observed (*28*,*29*).

5.2. *Sapindus mukorosii (reetha)*

Saponins isolated from *Sapindus mukorosii* (reetha) have shown most potent spermicidal activity. The saponins reported are derivatives of hederagenin namely mukurozi-saponins E_1, G, X, Y_1, Y_2, Z_1 and Z_2 (*30*). After incubation with saponins at 0.5 mg/mL for 1 min, the spermatozoa did not exhibit

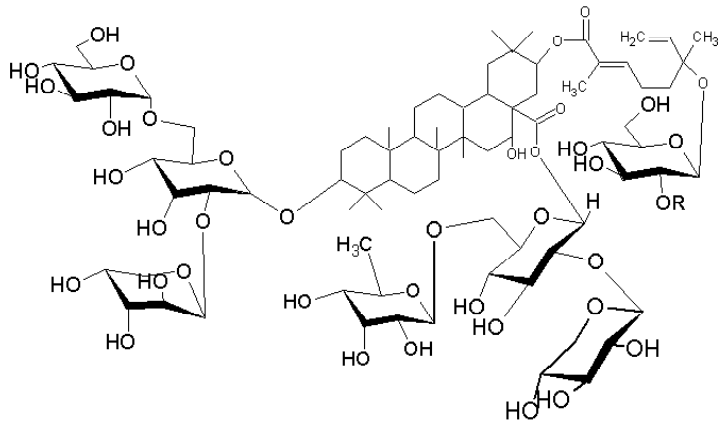

Figure 2. Structure of Acaciaside A and B. Acaciaside A: R= H; Acaciaside B: R= Xylose.

any significant morphological changes, though at the same concentration, immobilization of the sperms was observed. At higher concentrations of saponins (1-50 mg/mL) spermatozoa displayed marked disruption, vacuolation, vesiculation and erosion of the membrane covering the head region. Coiling of the tail was also noticeable with higher concentrations of saponins though no damage was evident under SEM in the flagellar region of the sperm (*27*,*31*). These saponins were formulated into a contraceptive cream named 'CONSAP'. This cream has completed Phase III clinical trials successfully in India (*32*).

5.3. *Molluga pentaphylla*

The ethyl acetate fraction of *Molluga pentaphylla*, a tropical herb contains an antifungal triterpenoid saponin, Mollugogenol-A (Figure 3) which has demonstrated spermicidal activity at 300 µg/mL. Electronic microscopic observation showed that the fragmentation or loss of plasma membrane, vesiculation of periacrosomal membrane and dissolution of the organelle as a whole are suggestive of sperm degeneration (*33*).

Other plants containing saponins, which have shown spermicidal activity, include *Phytolacca dodecadra*, *Calendula officinalis*, *Acacia caesia*, *Acacia concinnha*, T*rigonella foenum-graecum* (*34*), *Chenopodium album* (*35*) and *Cestrum parqui* (*36*). Saponins are naturally occurring and there is no report of their systemic toxicity. However, due to their interfacial tension reducing property they may alter the permeability of the vaginal membrane on frequent use. In addition, reduction in interfacial tension may infact, lead to decreased viscosity of the mucus and hence, result in an increased rate of transfer of spermatozoa through the vaginal mucosa. Therefore, it is necessary to critically evaluate these effects before advocating the use of saponins as spermicidal agents (*12*).

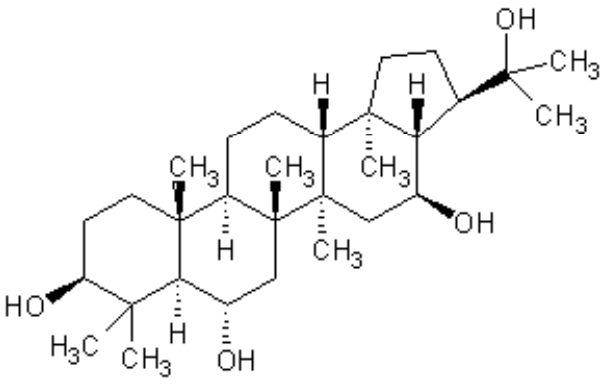

Figure 3. Structure of Mollugogenol-A.

6. Spermicides with additional antimicrobial activity

6.1. *Chlorhexidine*

A contraceptive method, which additionally protects against veneral infections, will be of immense value. One such compound being investigated for its spermicidal action is the antiseptic, chlorhexidine. The mechanism of the spermicidal action of chlorhexidine is not fully understood, however its antiseptic action is attributed to its high positive charge density resulting in non-specific binding to the negatively charged elements on the microbial cell wall. Disruption of cellular permeability, cell wall fluidity and altered metabolic activity has been suggested as a possible cause of the antiseptic action. Chlorhexidine shows spermicidal activity at the dose of 4.81 mg/mL within 20 sec, however hypersensitivity to chlorhexidine upon topical use has been reported (*37*,*38*).

6.2. *Magainins*

Magainins are class of peptides initially isolated from the skin of the African clawed frog, *Xenopus laevis*. Magainins A and G are two natural peptides having 23 amino acids and differ by only two substitutions, have been found to have a wide spectrum *in vitro* antimicrobial activity against gram positive and negative bacteria, fungi, and protozoa. They exhibit

spermicidal action, besides antimicrobial activity (*39*). Magainins are membrane active compounds and the decreased motility and viability of sperm has been observed in the presence of Magainins may be attributed to the loss of permeability of the plasma membrane, which leads to cell-death. Magainin-A was found to be more potent than Magainin-G. Intravaginal administration of magainin-A 200 µg to rats (*40*) and 1 mg to rabbits and monkeys once before mating resulted in 100% sperm immobilization (*41*,*42*). Magainin-A does not have overt cytotoxic properties and is safe for intravaginal application. It is also active against various STI-causing pathogens but not against HIV-1 and HIV-2. It is reported that effectiveness of magainin as a contraceptive *in vivo* is possibly due in part to the removal of cholesterol from sperm membranes (*43*).

6.3. *Nisin*

Nisin, a 34 amino acid, naturally occurring antimicrobial cationic peptide is known to be produced by bacteria *Lactococcus lactis*. Nisin has been used as a food preservative throughout the world and the World Health Organization (WHO) and US, Food & Drug Administration have conferred GRAS status to this peptide. At the dose of 300-400 µg, complete immobilization of human spermatozoa was observed within 20 sec. *In vivo* contraceptive efficacy studies in rats showed complete arrest of sperm motility and no pregnancy in any of the animals. At the contraceptive dose of 200 µg, Nisin did not alter the morphology of the vaginal epithelial cells, nor did it cause any histopathological lesions in the vaginal epithelium when administered intravaginally for 14 consecutive days. The mechanism by which Nisin exerts its rapid spermicidal action is not known. However, the existing evidence suggests that Nisin possesses an overall positive charge and interacts preferentially with anionic phospholipids. The sperm plasma membrane contains high concentration of phosphatidylglycerol, a strong anionic phospholipid moiety and thus, Nisin may have high affinity towards spermatozoa (*44*).

6.4. *Zidovudine derivatives*

Zidovudine *i.e.* 3'-azido-3'-deoxythymidine though lacks spermicidal activity by itself, its two novel phenyl phosphate derivates WHI-05 [5-bromo-6-methoxy-5,6-dihydro-3'-azidothymidine-5'-(*p*-methoxyphenyl) methoxyalaninyl phosphate] (Figure 4) and WHI-07 [5-bromo-6-methoxy-5,6-dihydro-3'-azidothymidine-5'-(*p*-bromophenyl) methoxyalaninyl phosphate] (Figure 5) have been identified to exhibit potent anti-HIV and spermicidal activity (*45*,*46*). They are dual-functional microbicides lacking detergent-type membrane toxicity, which would have advantages over

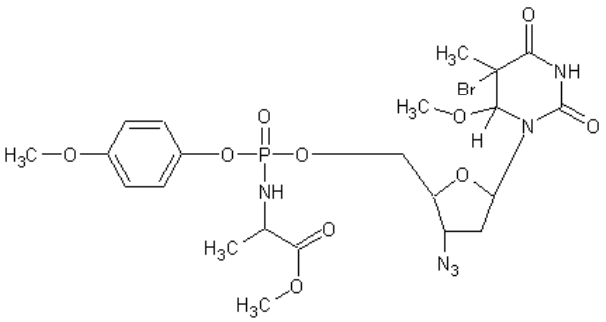

Figure 4. Structure of WHI-05.

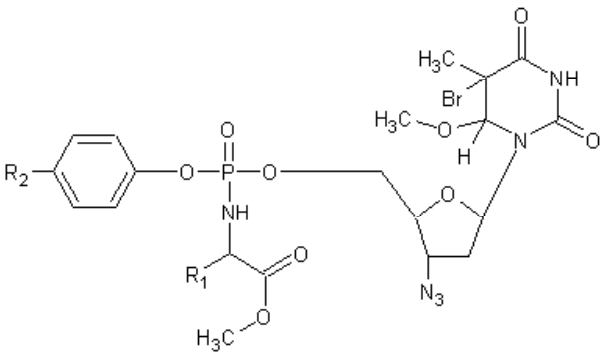

Figure 5. Structure of WHI-07. R_1= CH_3; R_2= Br.

the currently available vaginal microbicides. Unlike, N-9, the spermicidal activity of WHI-05 and WHI-07 was not associated with cytotoxicity to reproductive tract epithelial cells (*47*,*48*). A repeated intravaginal exposure of gel microemulsion formulations of WHI-05 and WHI-07 in mice and rabbits indicated these are non-cytotoxic and lacks inflammation-inducing properties (*49*,*50*). D'Cruz *et al*. also demonstrated that an intravaginal application of 2% WHI-07 *via* a gel microemulsion in rabbit model resulted in marked contraceptive activity (*51*).

6.5. *C31G*

C31G, a spermicide composed of an equimolar mixture of *n*-dodecyl-dimethylamine-*N*-oxide (C_{12}-N-O) and *N*-(*n*-dodecyl), *N*-dimethyl-glycine (C_{12}-betaine) offers a potential alternative to nonoxynol-9, both as a spermicide and as a microbicide/virucide. C31G has shown *in vitro* activity against a large number of gram-negative, gram-positive bacterial strains and anti-fungal properties. It is a potent virucidal agent with activity against HIV and herpes simplex virus (*52*). A phase I double-blind randomized study of 1.2% C31G with hydroxylethyl cellulose (HEC) suggested that physical epithelial changes after 7 consecutive days of product use were similar to changes seen with a marketed 2% nonoxynol-9 product (Gynol-II). The subjective symptoms of genital burning or heat, however, were much greater with the C31G HEC product, which limits its usefulness (*53*).

7. Antiliquefying agents

The active antiliquefying agents immediately coagulate ejaculated semen, possibly through a denaturing effect on the glycoproteins present in coagulated material. Highly effective antiliquefying property has been exhibited by mercury (2.7 mg/mL), nitrophenols (6.9 mg/mL), sodium naphthyl phosphate and tannic acid. A combination of antiliquefying and a potent spermicidal agent may offer highly promising approach towards vaginal contraception. However, the safety index of the currently evaluated antiliquefying compounds is too low to permit their use in pharmaceutical formulation for *in vivo* use (*54*).

8. Calcium ion and sperm motility

Calcium ions have an apparently paradoxical effect on sperm motility. Hong CY *et al*. states that in epididymis, calcium ions stimulate immature sperms but in ejaculated semen, calcium ions inhibit sperm motility. Thus calcium chelators such as ethylene glycol-bis β-aminoethyl ether *N,N,N',N'*-tetraacetic acid (EGTA) and ethylenediamine tetraacetic acid (EDTA), as well as calcium antagonists such as diltiazem, flunarizine and verapamil stimulate sperm motility in ejaculated human semen (*55*).

However, Lee C *et al*. stated that a decrease in calcium ion concentration in semen will inhibit sperm motility. After exposure to EDTA, the calcium ion concentration in semen was found decreased with increasing EDTA concentration. Thus EDTA appears to exert the spermicidal activity by modulating calcium ion concentration in semen. EGTA 5.5 mg/mL, EDTA 5 mg/L showed activity within 2 min (*56*).

The sperm membrane is reported to possess a Na^+-Ca^{2+} exchanger and a Ca^{2+}-ATPase pump. Both these systems play a vital role in extrusion of Ca^{2+} from the sperm cell. 2',4'-dichlorobenzamil hydrochloride (Benzamil), has been reported to inhibit both the above systems and thus benzamil exhibits spermicidal activity due to the elevation of intracellular Ca^{2+}. Benzamil at 2.0 mM concentration showed 100% immotility at 60 min. But when used in combination with propranolol (2.0 mM), the spermicidal activity was seen within 8 min. Propranolol is also reported to produce sperm death due to an increase in intracellular Ca^{2+}. This action may be due to its membrane stabilizing property and not related to its β-blocking property (*57,58*). When 2',4'-dichlorobenzamil hydrochloride (DBZ) was combined with any one of the three H_2-receptor antagonists, cimetidine, ranitidine and famotidine, the time required to produce complete loss of sperm viability was found to be reduced by minimum of 2.7, 1.9 and 3.4 fold, respectively. The elevation of intrasperm Ca^{2+} by H_2-receptor antagonists can be attributed to their ability to inhibit Na^+-K^+ ATPase enzyme system that is reported to be present on the sperm membrane. Thus the rate of increase of intrasperm Ca^{2+} was found to be faster when DBZ was used in combination with any H_2-receptor antagonists (*59*).

9. Ionophores

Ionophores are compounds that form lipid soluble complexes with specific cations and act as vehicles for transporting these cations across biological membrane. The calcium ionophore, A23187, increases the intracellular calcium concentration and inhibits human sperm motility at the concentration of 20 μM within 120 sec. However, local effects of ionophores on vaginal tissue and their systemic effects after absorption are yet to be evaluated (*60*).

10. Miscellaneous agents

10.1. *Gossypol*

Gossypol, a disesquiterpene aldehyde (Figure 6) isolated from the seeds of cotton (*Gossypium* species) plant, is reported to be a spermicidal agent (*61*). The concentration required to immobilize 100% spermatozoa within 20 sec is 40 mg/mL (*62*). Gossypol inhibits sperm motility by blocking ATP production and utilization. It acts on mitochondria, suppressing oxygen consumption, inhibiting the pyruvate dehydrogenase and ATPase activities and probably on the motility apparatus by blocking dynein ATPase activity and preventing protein phosphorylation (*63*).

10.2. *Lyophilized Aloe barbadensis*

Aloe barbadensis, one of the worldwide botanicals, has been used for health purposes for thousands of years and comes in a variety of forms including gel and lyophilized powder. Fresh gels are not used intravaginally as they are unstable and also contain

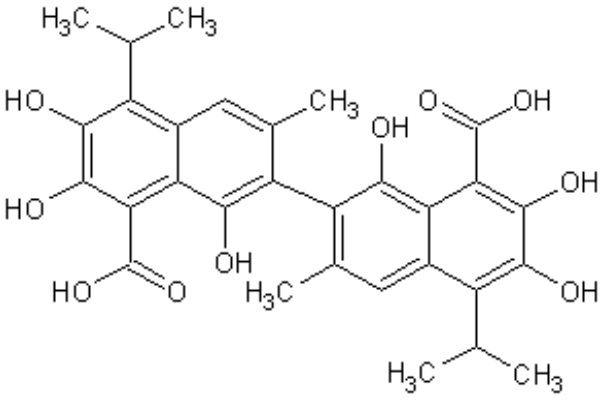

Figure 6. Structure of Gossypol.

sugars, which may accelerate vaginal infections. Lyophilized *Aloe barbadensis* does not contain sugars but eleven different mineral elements in different concentrations, which have shown a toxic effect on the tail of spermatozoa leading to rapid immobilization of spermatozoa. It was seen that the spermatozoa were intact, but their tails were curled after being exposed to lyophilized *Aloe barbadensis* at 100 mg/mL concentration within 30 sec. Rabbit vaginal irritation study showed no irritation of vaginal epithelium after application of 100 mg/mL lyophilized *Aloe barbadensis* for 10 days (*64*).

10.3. *Azadirachta indica*

The neem tree, *Azadirachta indica* is indigenous to the Indian subcontinent. Neem oil, an oil extracted from the seeds of the neem tree, has been found to possess strong spermicidal activity. By the process of hydrodistillation, the volatile fraction of neem oil has been isolated and coded as NIM-76. A concentration of 25 mg/mL of the compound was found to achieve total spermicidal effect in 20 sec. Vaginal irritation study conducted in rabbits, by intravaginal application of 15 mg of NIM-76 in 2 mL of gelatin jelly for 10 days showed no irritation to the vaginal mucosa (*65*). Khillare B has revealed that the aqueous extract of old and tender neem leaves is a potent spermicide. The minimum effective concentration required to kill 1 million sperm in 20 sec was 2.91 mg and 2.75 mg for tender and old leaf extract, respectively (*66*).

10.4. *Allium sativum*

Garlic and its active principle, allitridium (Figure 7) possess bacteriostatic and antimycotic action. Allitridium showed complete immobilization of sperms from human and animals within 20 sec at 7.5 mg/mL and within 3 min at 1.5 mg/mL. Allitridium (7.5 mg/mL) showed no vaginal irritation reaction or other side effects. It had no bacteriostatic action on the lactic acid bacilli, so it would not interfere with the growth of the bacilli in the vagina (*67*).

$$CH_2=CH-CH_2-S-S-S-CH_2-CH=CH_2$$

Figure 7. Structure of Allitridium.

10.5. *Curcuma longa (Turmeric)*

Curcumin (diferuloyl methane) (Figure 8), a yellow pigment present in the rhizomes of turmeric and related species and used as a spice, has a wide array of pharmacological and biological activities. Studies have demonstrated that curcumin has anti-tumor, anti-inflammatory and anti-infective activities. Curcumin

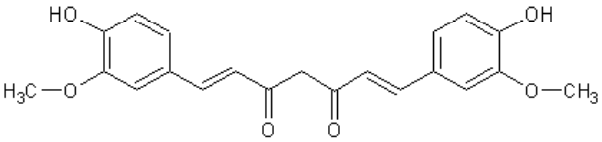

Figure 8. Structure of Curcumin.

has also shown to inhibit the integrase activity of the HIV. When curcumin is used in the concentrations of 30 μg/mL and 100 μg/mL, the human sperm motility was reduced to 53.4% and 4.1% after 120 min. A total 100% immobilization was achieved with a dose of 300 μg/mL at the end of 60 min. Curcumin-induced changes in sperm mitochondrial transmembrane potential indicate that this flavanoid may interfere with sperm energy metabolism. Curcumin in micromolar concentrations inhibits the protein kinase C, which is present in human sperm and is believed to play a role in modulating human sperm flagellar movement (*68*).

10.6. *Stephania hernandifolia and Achyranthes aspera*

A composite extract of the leaves of *Stephania hernandifolia* and the roots of *Achyranthes aspera* were prepared in a ratio of 1:3 and evaluated for spermicidal activity at different concentrations ranging from 0.04 to 0.32 g/mL. Concentration of 0.08 g/mL of the extract affected the motility and at a concentration of 0.16 g/mL, the sperm motility was reduced to 20% immediately within 20 sec. At the concentration of 0.32 g/mL complete sperm immobilization was observed within 2 min after application of the extract. The hypo-osmotic swelling of these sperms was reduced significantly at this highest concentration, indicating that the crude extract may probably cause injury to the sperm plasma membrane. A low concentration of 0.04 g/mL was found to be ineffective (*69*).

10.7. *Carica papaya seed extracts*

The chloroform extract, the benzene chromatographic fraction of the chloroform extract and its methanol and ethyl acetate subfractions and the isolated compounds ECP 1 and 2 and MCP 1 and 2 have shown a sperm immobilizing effect on human spermatozoa *in vitro*. Total inhibition of motility was observed within 20-25 min at all concentrations of all products. The SEM and TEM of spermatozoa showed membrane damage in the head as well as midpiece suggesting the mode of action appears similar to that of N-9 (*70*).

10.8. *Praneem polyherbal formulations*

A combination formulation developed as "Praneem polyherbal cream" which includes a purified extract from the dried seeds of *Azadirachta indica* (Neem) (250 mg/mL), extract from the pericarp of fruits of *Sapindus*

mukorosii (0.5 mg/mL) and quinine hydrochloride (3.46 mg/mL) has shown spermicidal activity in 20 sec. The formulation has shown high contraceptive efficacy in rabbits and in monkeys after intravaginal application. Also the formulation was found to be safe with no vaginal irritation when applied intravaginally for 30 days at a daily dose of 1 mL (*71*).

The Praneem polyherbal pessary and tablet formulated, includes purified ingredients from neem leaves, *Sapindus mukorosii* and *Mentha citrata* oil. The vaginal pessary has shown potent spermicidal action of human spermatozoa *in vitro* and high contraceptive efficacy was demonstrated in rabbits of proven fertility (*72,73*). Praneem polyherbal formulations have shown *in vitro* activity against HIV and sexually transmitted disease pathogens (*73*). Praneem vaginal pessaries and tablets were found to be safe for once daily intravaginal use consecutively for 7 and 14 days in healthy women volunteers (*74,75*).

10.9. *Parabens*

Parabens are commonly added in food, beverages, pharmaceuticals and cosmetics as antifungal preservatives. Methyl paraben, ethyl paraben, propyl paraben and butyl paraben have shown potent spermicidal activity at the concentrations of 6, 8, 3, and 1 mg/mL, respectively (*76*).

10.10. *Zinc acetate*

Zinc acetate at 10 mg/mL concentration has shown spermicidal activity within 30 sec, while the other zinc salts such as zinc gluconate, zinc sulfate and zinc chloride are not spermicidal at the same concentration. It is probably that zinc ion and acetate decrease the availability of oxygen to sperm, which leads to immobilization. Transmission electron microscopy of zinc acetate treated human spermatozoa showed the most visible changes in the mitochondria of the middle portion of the tail. There was a reduction in the electrodensity of mitochondria of the spermatozoa but the sheath was present. Vaginal irritation studies in rabbits with continuous administration of 4 mg zinc acetate/kg or 8 mg zinc acetate/kg for 10 days caused no irritation of highly sensitive rabbit vaginal epithelium. In addition zinc has also been reported to be beneficial to wound healing (*64*).

10.11. *Gel microemulsions*

Microemulsions are thermodynamically stable, isotropically clear dispersions of water, oil, and surfactants with potential as drug-delivery vehicles. D'Cruz O *et al*. has formulated novel submicron (30-80 nm) particle gel microemulsion (GM) formulations GM-144 and GM-4. GM-144 prepared from seven non-toxic pharmaceutical excipients (propylene glycol, Captex 300, Cremophor EL, Phospholipon 90G, Rhodigel, Pluronic F-68 and sodium benzoate) was found to show rapid sperm-immobilizing activity in human semen in less than 30 sec (*77*). GM-4 formulation containing eight pharmaceutical excipients (Captex 300, Cremophor EL, Phospholipon 90G, Propylene glycol, PEG-200, Seaspan carrageenan, Viscarin carrageenan and sodium benzoate) exhibited potent spermicidal activity in less than 2 min (*78*). In standard rabbit model, GM-144 and GM-4, when tested as a vaginal contraceptive, GM-144 was as effective as the commercially available N-9 formulation (Gynol II) and GM-4 was far more effective than Gynol-II. No toxic effect was observed on the vaginal mucosa of rabbits after daily exposure for 10 days (*77-80*).

10.12. *Vanadocenes*

Spermicidal organometallic complexes of vanadium (IV) with bis(cyclopentadienyl) rings or vanadocenes are a new class of experimental contraceptive agents. Vanadocenes are reported to have rapid, potent and selective sperm immobilizing activity (SIA). Vanadocenes elicited potent SIA at nanomolar to micromolar concentrations. The SIA of representative vanadocenes was 400-fold more potent than that of N-9. Vanadocenes dihalides immobilized human sperm in semen within 15 sec without affecting the sperm membrane integrity or viability of normal human vaginal or cervical epithelial cells. These features of vanadocenes fundamentally differ from those of currently used membrane-active detergent-type spermicides that are cytotoxic to genital tract epithelial cells at spermicidal concentration. The lack of detergent-type membrane toxicity of spermicidal vanadocenes may have particular clinical utility as a new class of contraceptive agents. Spermicidal activity of vanadocenes were shown to be mediated by a unique mechanism involving membrane intercalation that was independent of dynein adenosine triphosphatase activity, protein tyrosine phosphatase activity, and the phosphocreatine/creatine kinase system. Among the 45 vanadocenes that were synthesized and evaluated for human spermicidal activity vanadocene acetylacetonato monotriflate (VDACAC) (Figure 9) and vanadocene dithiocarbamate (VDDTC) (Figure 10)

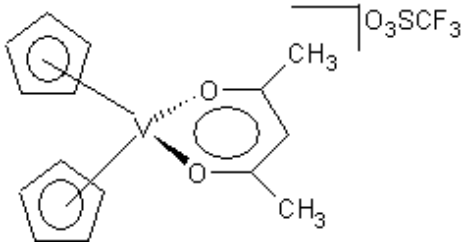

Figure 9. Structure of vanadocene acetylacetonato monotriflate (VDACAC).

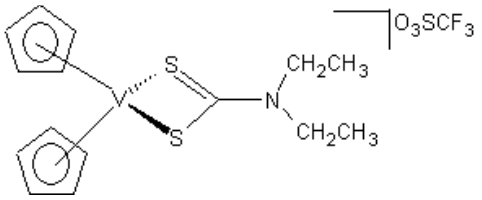

Figure 10. Structure of vanadocene dithiocarbamate (VDDTC).

were the most stable and potent spermicidal vanadocenes (*81-88*). Repeated intravaginal application of gel formulations of VDACAC and VDDTC did not induce local inflammation, toxicity or retention of vanadium in the mice and rabbit vaginal irritation model. D'Cruz OJ *et al*. demonstrated that the intravaginal application of a 0.1% VDDTC in rabbits and pigs results in remarkable contraceptive activity (*89-92*).

11. Conclusion

This summary of the spermicides pipeline and complementary research clearly shows that much progress has been made in the last decade. Laboratory and clinical research has been complemented by a growing body of research and literature on spermicides acceptability, harm reduction and protection strategies, and potential markets. In recent years, attitudes toward spermicides have generally become more positive in response to public and nonprofit initiatives to address these barriers. However, many challenges remain, including the need for a significant increase in investment to accelerate product development and complementary research, and to plan for availability and access once effective spermicides are available.

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