REVIEW
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LITERATURE
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1.1.0 Immunological competence of the female reproductive tract

It is now well established that the female reproductive tract in mammals is not an immunologically privileged site and it possesses both affector and effector components of the immune system. The presence of lymphatics in endometrium was demonstrated in mice by Fabian (1976). Recent reports, however show that the mucosal epithelium lining of the uterus lacks an intrinsic lymphatic system, although the deeper layers, including the myometrium have a rich supply of lymphatic vessels drained by regional lymph nodes (Head, 1987a). In addition, the presence of immunocompetent cells have been reported in the human endometrium (Sen and Fox, 1967; Kamat and Isaacson, 1987). These cells exhibit cyclic distribution, representing 10-15% of stroma during follicular phase and 20-25% in the late secretory phase. The major leukocyte population are T cells and macrophages. The T $\gamma^+$ suppressor/cytotoxic population are predominant in the stroma as well as in the epithelial layer. NK cells, have been shown to be present, scattered singly in the stroma. Recently, Laguens et al. (1990) have demonstrated the presence of HLA-DR positive antigen presenting cells in the stroma of human endometrium. These cells represent 13-25% of the stromal cells in the proliferative and 16-43% in the secretory endometrium and their distribution is apparently under hormonal control. Majority of these cells do not represent macrophage lineage, indicating that they are either a special population of connective tissue stroma cells able to express HLA-DR or reticular dendritic cells (Laguens et al, 1990).

The presence of leukocyte population, including Langerhans cells, macrophages, natural killer cells and T and B lymphocytes have also been reported
in the reproductive tracts of experimental animals (Head, 1987a). In their study, Parr and Parr (1991) showed that Langerhans cells (LCs) in the epithelium of mouse uterus expressed Ia and common leukocyte antigen and were found to be a phenotypically heterogeneous population. T lymphocytes of both helper and cytotoxic/suppressor types were also reported to be present in the epithelium, sometimes in close association with LCs, but NK cells were not observed. The stroma of the vagina and cervix contained LCs and macrophages but few T lymphocytes and no B cells, NK cells or lymphoid nodules. The presence of Ia antigen bearing antigen-presenting cells has been demonstrated by Head et al (1987b) in the rat uterine endometrium. A functionally active complement system has also been reported to be present in the uterine secretion of mouse (Jin et al, 1991).

Many studies have been done to localize immunoglobulins and plasma cells in the female reproductive tract of different animal species. Parr and Parr (1985) showed that Ig A and Ig G were located in plasma cells in the endometrium of uterus as well as in the oviduct of mouse. No Ig M was detected in any part of the murine reproductive tract. The majority of plasma cells secreted Ig A type of immunoglobulins. Similar results have been found in human reproductive tract where Ig A was the predominant immunoglobulin produced. The reproductive hormones have been shown to play an important role in regulating the number of plasma cells at different times of the cycle (Rebello et al, 1975; McDermott et al, 1980; Murdoch et al, 1982; Sullivan et al, 1984).

Experimental evidence substantiating the immunocompetence of the female reproductive tract was presented by Beer and Billingham (1974), by their demonstration that histocompatible skin grafted into the uterine wall survived with remarkable frequency compared to histoincompatible allogenic skin graft which
were rejected in the same fashion as if they had been transplanted to more conventional site. The rejection phenomenon was shown to be cell mediated, since skin allografts in the uterus of immunologically tolerant rats were promptly rejected when specifically sensitized immune cells were passively transferred into recipient host (Beer and Billingham, 1976). More recently, studies have shown that exposure of genital mucosa to microbial antigens leads to leukocytic infiltration (Ogra et al, 1981; Parr and Parr, 1988a) and induction of antigen-specific Ig A antibody response (Waldmann et al, 1972; Ogra and Ogra, 1973). Studies have also demonstrated that leukocytes can be attracted to the uterus and effective immune response can occur in this location (McAnulty and Morton, 1978; Anderson and Alexander, 1979). One of these studies indicated that glycogen induced uterine leukocytosis effectively terminated pregnancy before and during implantation stage. Trial of various routes to raise specific antibody response showed that in the reproductive tract Ig A is the predominant and effective immunoglobulin (McDermott et al, 1979; Parr et al, 1988b; Parr and Parr, 1990). Intrauterine immunization with mycobacteria induced local reactivity that could be shown on secondary intrauterine challenge, but no detectable systemic immunity (Targowshi, 1984). However, there are a few reports that systemic immunity could develop following intrauterine exposure to hapten-protein conjugate (Lande, 1981). The reason for this maybe the longer retention of antigen within the uterine lumen, use of alum precipitated antigen for immunization and / or the repeated intrauterine exposure of the females to the antigen.
1.2.0 Immunological response of female reproductive tract to spermatozoa during normal reproductive process.

The reproductive tract of the female undergoes repeated inoculation with hundreds of millions of spermatozoa - highly specialized and immunogenetically alien, together with other cell types, including leukocytes suspended in complex protein containing seminal plasma secreted by specialized accessory reproductive organs of the male. The possible immunological effects of this process have been a subject of a great deal of speculation.

Studies by Austin (1957, 1960) have shown that in a large number of species including mice, rats, rabbits, bats, moles, hedgehogs, guinea pigs and bats, coitus leads to leukocytosis in the female reproductive tract, followed by phagocytosis of the sperms. These leukocytes as well as the epithelial cells of the mucosal lining of the genital tract were shown to play an active part in sperm phagocytosis (Philips and Mahler, 1975). Investigation of the rabbit vaginal response to semen and to sperm free seminal plasma revealed that the leukocytic response is caused by semen (Philips and Mahler, 1977). Tyler (1977), on the other hand found little leukocytic response to copulation with vasectomized bucks, hence concluding that leukocytosis seemed to be triggered by spermatozoa rather than seminal plasma. Pandya and Cohen (1983) showed that women also respond by leukocytosis to cervical deposition of spermatozoa. The primary function of the leukocytes maybe through sperm selection by phagocytosis or the mopping up of introduced bacteria. Since the study was carried out in women who were being artificially inseminated and some who consequently became pregnant, it was concluded that this leukocytosis is a physiologic response of the cervix and not a pathologic reaction. These results were reaffirmed by recent study (Thompson et al, 1991) showing leukocytic influx across the human uterine cervix.
following introduction of semen samples. The finding of Parr and Parr (1988a) supported the view that the neutrophils in the uterus following mating played an important role in phagocytosing the bacteria and returning the uterus to an aseptic state before implantation.

Allogenic spermatozoa deposited normally into the mouse uterus during coitus also induce hypertrophy and hyperplasia in the para-aortic lymph nodes which drain the mouse uterus (Beer and Billingham, 1976). Similar striking hypertrophy of the nodes draining the uterus can be observed in pregnant rats, mice, hamsters and women bearing genetically alien features. This phenomenon has been correlated with increased levels of immunoglobulin secreting cells in the lymph nodes draining the uterus, as well as potentiated levels of T-cell responses (Dresser, 1991). All these reports emphasize the fact that although there is an immune response in the female reproductive tract following coitus, this phenomenon does not lead to immunity against sperm even after repeated exposure. In fact there is evidence that this immune response is beneficial to the development of the fetus (Wegmann, 1988; Guilbert et al 1991). However, the mechanism underlying the immune protection provided to sperm during normal reproductive process still remains unclear, and has been the subject of widespread speculation among many workers. The mechanism appears to be a complex phenomenon involving immunosuppressive factors in the seminal plasma (Saxena et al, 1985) and uterine secretions (Segerson, 1988) and suppressor cells in the uterine endometrium (Clark et al, 1984). Immunosuppressive factors produced by the reproductive tissues themselves (Alexander and Anderson, 1987; Castilla et al, 1990), the placenta (Menu et al 1991) as well as the embryo (Sheth et al 1991; Daya and Clark 1986) have also been shown to facilitate embryo implantation.
1.3.0 Evidence that CMI reaction affect female reproductive functions

As is evident by the data available that lack of immune response against sperm is necessary for successful pregnancy. There is also growing evidence that the breakdown of this immune protection affects fertility. There have been numerous studies of antisperm CMI responses in infertile women. Studies by Marcus et al (1973), Mettler and Schirwani (1975), Soffer et al (1976) and McShane et al (1985) have documented CMI responses to sperm. Cellular sensitization was measured in terms of in vitro macrophage migration inhibitory activity or leukocyte inhibitory factor or lymphocyte transformation tests in a large number of women with or without antisperm antibody. The results provide evidence that significant number of infertile women had cellular immunity to sperm-specific antigen. Recent studies (Haney et al, 1983; Xu et al, 1987) have shown that infertile women have large number of activated T cells in the endometrium. Large number of oviductal macrophages were also found in such patients, supporting the view that peritoneal macrophages migrate into the oviducts and interfere with fertilization by phagocytosing sperms in vivo (Haney et al, 1983). Studies demonstrating that intraperitoneal transfer of hyperactivated but not basal state macrophages significantly inhibited fertilization (Steinleitner et al, 1991) reaffirm the earlier findings. Significantly enhanced number of leukocytes have been found in the cervicovaginal environment of asymptomatic women with cervical factor infertility (Wah et al 1990). These data suggest that cervicovaginal leukocytes may play an important role in infertility since soluble products of leukocytes are known to adversely affect reproduction.

Endometriosis patients who have about 20 times greater risk of infertility (Strathy et al, 1982) were observed to have large number of activated macrophages and T cells in their peritoneal cavity (Haney et al, 1981; Halme and Hall, 1982;
Chacho et al, 1986; Hill et al, 1989a). The soluble products like IL-1 secreted by these cells have been implicated as factors contributing to their infertility (Fakih et al, 1987; Anderson et al 1991, Haimovici et al, 1991). Gentry et al (1989), failed to demonstrate significant antisperm antibody in the peritoneal fluid of patients with endometriosis, further confirming the role played by cell mediated immunity in infertility.

Several potential mechanisms of CMI-induced infertility have been revealed. As mentioned above, soluble products of activated lymphocytes and macrophages including lymphokine gamma interferon and monokine TNF, have been shown to affect human sperm motility (Hill et al, 1987a) and fertilization events as measured by hamster egg penetration test (Hill et al, 1989b). Therefore, activated macrophages and lymphocytes residing in various regions of female reproductive tract could affect sperm function through release of cytokines and other toxic factors such as free oxygen radicals (Nathan, 1980). Macrophage and lymphocyte rich peritoneal fluids from women with endometriosis have also been shown to adversely affect sperm function (Halme et al, 1982). A variety of lymphokines and monokines have been studied for effects on early embryonic development. Gamma-interferon has been reported to affect development of early mouse embryo (Hill et al, 1987b; Haimovici 1991) and fetal cells at later stages of gestation (Drasner et al, 1979). Granulocyte, macrophage- colony stimulating factor may also adversely affect early embryonic development (Hill et al, 1987b; Anderson et al, 1991). The effect of monokine IL-1 on embryonic development is still controversial. Fakih et al (1987) reported its possible role in infertility while Schneider et al (1987) have reported that it is not toxic to mouse embryo development, while Hill et al (1987b) have found it embryotoxic only at a very high concentration.
Lymphocyte and macrophage products may also affect the immunogenicity and viability of trophoblast cells. A number of laboratories have reported enhancement or induction of class I MHC antigens on cells of trophoblastic lineage in the presence of interferon (Head et al, 1987). Induction of MHC antigens on trophoblast by lymphokines could be a significant mechanism in CMI-mediated spontaneous abortion since paternal MHC antigens could serve as targets for maternal cytotoxic T cells as well as immunological responses.

1.4.0 Experimental induction of local immune response against spermatozoa in female reproductive tract.

Studies were carried out to induce infertility by immunization of spermatozoa in different species like mice, guinea pigs and even humans (Katsh, 1959; McLaren, 1960) as far back as 1950's and 1960's. Immunization of female animals of various species with extracts of sperms or mature testis resulted in a significant inhibition of fertility. The route of immunization was systemic and the antifertility effects were attributed to antispermatozoal antibody developed after immunization. However, no correlation was observed between levels of antisperm antibody and the degree to which fertility was impaired (McLaren, 1960), leading to the speculation that cell mediated immunity rather than circulating antibody is responsible for infertility. In 1984, Allardyce reported that intragastric administration of live epididymal rat sperm to adult female virgin rats resulted in short to long term infertility related to the appearance of antisperm antibody of Ig A type in genital tract secretions preceding mating. These findings support the concept of a common mucosal immune system (McDermott et al, 1980) in that an immune response to non-self tissue antigens generated in gut associated lymphoid tissue maybe expressed at other mucosal sites and that such a response to sperm antigens in the genital tract may diminish fecundity. Shelton and
Goldberg (1986), also reported similar findings with their sperm specific isozyme lactate dehydrogenase C4 (LDH-C4). After intrauterine immunization with LDH-C4, female mice were found to secrete Ig A antibodies specific for LDH-C4 in their uterine fluids. These animals demonstrated a lower pregnancy rates than controls. The conclusion therefore was that induction of a local immune response offers an alternative to systemic immunization for administrating a contraceptive vaccine.

Lately, efforts have been made by workers to induce cell-mediated immune responses to sperm antigen. Naz and Mehta (1989) showed that purified fertilization antigen (FA-1), protamine and lithium diiodosalicylate (LIS) - solubilized sperm preparations activated presensitized lymphocytes to secrete soluble mediators that activated macrophages and significantly inhibited sperm motility and embryonic development. Alexander and Anderson (1979) have demonstrated that non-specific leukocytosis in the uterus in response to inert agents like glycogen can be utilized to effectively terminate pregnancy before and during implantation stage. The most common example of non-specific CMI response to sperm or embryo in the genital tract is the intrauterine contraceptive device (IUDs) which act as physical irritant and induce a local inflammatory reaction. In fact, IUDs recovered after long usage have been found to contain large number of macrophages, lymphocytes and neutrophils and the T lymphocytes scrapped from the IUDs have been shown to elicit a significant in vitro proliferative response to non-specific mitogens (Ogra et al, 1981).

1.5.0. Herbal Products In Contraception

The concept of using herbal products for contraception is not a new one. Ancient texts in the Indian, Antharva Veda, 2500 B.C. and Chinese, Sheng Nung
Pents as Ching, 1122 B.C., systems of medicine mention plants possessing antifertility properties. The use of plants as emmenagogues, abortificients and as local contraceptives was well known to physicians of India, in ancient times. Even today, nomadic and aboriginal tribes in India are alleged to use plant contraceptives to limit their family size. Research has been carried out intensively in this field from 1945 when the antifertility effect of *Lithosperm rederale* reportedly used by American Indians for contraception was investigated. The WHO has set up a Task Force on plants for fertility regulation, the strategic plan of which is to identify novel drug prototypes found in plants which have been alleged to have fertility regulating properties. Compounds that are being sought in particular are those orally active non-steroidal, non-estrogenic, safe and effective for prevention or disruption of implantation in women and those that will inhibit spermatogenesis or interfere with sperm maturation in man (Griffin, 1988). Recently, data on African (Kokwaro, 1981), Haitian (Weniger et al, 1982), Indian (Kamboj, 1988), Korean (Woo et al, 1981), Russian (Kharkhov and Mats, 1981) and Chinese (Kong et al, 1986) plants pertinent to fertility regulation have been summarised. These include a large number of anti-implantation, abortifacient agents in female as well as male contraceptives. In India itself according to Kamboj (1988) about 120 plant species belonging to 103 genera and 54 families have been mentioned as emmenagogues or abortificients in ancient Indian literature. Of these, 48 plant species covering 43 genera and 32 families have been tested for anti-implantation activity and 13 plants for abortifacient activity.

Only a few plants, reported to possess antifertility activity, have reached the stage of scientific clinical evaluation (Chaudhury, 1986). These plants are:

a) Gossypol from *Gossypium herbaceum*
b) *Montanoa tomentosa*
c) *Embelia ribes*

d) *Hibiscus rosasinensis*

e) *Vicoa indica*

1.6.0. Neem (*Azadirachta indica / Melia azadirachta*)

Neem is a large evergreen tree, 12-18 m in height and 1.8-2.4 m in girth, with long spreading branches forming a broad crown. It is a native of Indo-Malaysian region. The species occurring in India is *Azadirachta indica* A. Juss. syn. *Melia azadirachta* Linn. It is commonly found throughout the greater part of India, growing wild and often cultivated.

Almost every part of the tree finds application in indigenous medicines. Neem extracts have been reported to possess anti-diabetic (Chakraborty *et al*, 1984), anti-bacterial (Singh & Sastry, 1981) and anti-viral (Babbar *et al*, 1982) properties and have been used successfully in cases of stomach worms and ulcers. The stem, root bark and young fruits are reported to possess astringent, toxic and anti-periodic properties. The bark is reported to be beneficial in malaria fever and useful in cutaneous diseases.

The gum exuded by the bark is stimulant, demulcent and toxic and is useful in catarrhal and other affectations.

The twigs and leaves can be fed to cattle in conjunction with other feeds. It increases the secretion of milk as well as being carminative and aiding in digestion.

The tender leaves along with *Piper nigrum* Linn., are found to be effective in intestinal helminthiasis (*Wealth of India, 1985*). Fresh, mature leaves along with
the seeds of other plants are used to prepare a very effective medicine for leucoderma. A 10% aqueous extract of tender leaves is reported to possess antiviral properties against vaccinia-, vacuola-, foulpox- and New Castle disease virus. The essential oil from the leaves possess marked antibacterial properties and inhibit growth of *M. tuberculosis*, *Micrococcus pyogenes*-*aureus*, *Salmonella paratyphi*, *S. typhi*, *Vibrio cholrae*. The leaves are also used as insect repellents and to control nematodes (Wealth of India, 1985).

The fruit is used as a tonic, purgative, emollient and as an antihelminthic. It is beneficial in urinary diseases and treatment of piles. The dry fruits are bruised in water and employed to treat cutaneous diseases. The pulp water when sprayed, protects crops from locusts (Wealth of India, 1985).

The fruits are collected during April-August. They are sun-dried and stored till October-December or even later. Storing of seeds for three months is necessary for optimum yield of oil. The seed on average comprises 44.7% kernel and 55.3% shell.

The seeds contain azadirachtin, the most extensively studied constituent of neem seeds, which inhibits feeding of locust (*Schistocerca gregaria*) at the dose of 40 ug/litre and kills nymphs of *Periplanata americana* at the dose of 0.75 mg/kg body weight. Extracts of the seed act as gustatory repellents and have anti-feeding activity in insect species harmful for grains.

**OIL**: The kernels yield a greenish yellow to brown, acrid, bitter fixed oil (40-48.9%) known as oil of Margosa, having a strong, disagreeable odor.

The oil has many therapeutic uses. Most of the medicinal properties are attributed to the bitter principles and odorous compounds. It is a useful remedy in
some chronic skin diseases and ulcers. It is used for external application for rheumatism, leprosy, sprain, dental and gum problems. It possesses anti-septic and anti-fungal activity and has been found to be active against gram-positive and gram-negative organisms.

The refined and purified oil has following characteristics (The Pharmacopoeia of India, 1966):

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity(^{30})</td>
<td>0.9087</td>
</tr>
<tr>
<td>nD 30</td>
<td>1.4612</td>
</tr>
<tr>
<td>Iodine value</td>
<td>66.4</td>
</tr>
<tr>
<td>Saponification value</td>
<td>290.9</td>
</tr>
<tr>
<td>Unsaponified matter</td>
<td>0.8 %</td>
</tr>
</tbody>
</table>

The fatty acid composition of the oil is as follows (Gupta and Mitra, 1953)

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic</td>
<td>0.2 %</td>
</tr>
<tr>
<td>Palmitic</td>
<td>16.2 %</td>
</tr>
<tr>
<td>Stearic</td>
<td>14.6 %</td>
</tr>
<tr>
<td>Arachidic</td>
<td>3.4 %</td>
</tr>
<tr>
<td>Oleic</td>
<td>56.6 %</td>
</tr>
<tr>
<td>Linoleic</td>
<td>9.0 %</td>
</tr>
</tbody>
</table>
The component glycerides are:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitodistearin</td>
<td>0.2%</td>
</tr>
<tr>
<td>Oleopalmistearin</td>
<td>20.3%</td>
</tr>
<tr>
<td>Oleodistearin</td>
<td>1.6%</td>
</tr>
<tr>
<td>Palmito-oleolinolein</td>
<td>6.6%</td>
</tr>
<tr>
<td>Palmitodiolein</td>
<td>26.3%</td>
</tr>
<tr>
<td>Stearo-oleolinolein</td>
<td>3.6%</td>
</tr>
<tr>
<td>Stearodiolein</td>
<td>24.9%</td>
</tr>
<tr>
<td>Linoleodiolein</td>
<td>16.5%</td>
</tr>
</tbody>
</table>

The bitter principles of the oil have been obtained by extraction with alcohol in a yield of 2%. These include proto-meliacins, meliacins (limonoids or tetranortriterpenoids, tetranortriterpenoid-hydroxybutenolides, ring-c-seco-tetranortriterpenoids and ring-c-seco-tetranortriterpenoid-hydroxybutenolides), pentanortriterpenoids, a hexanortriterpenoids and norterpenoidal constituents (Siddiqui et al, 1988).

Nimbidin (yield 1.2-1.6%), the main constituent, is highly bitter. Besides nimbidin two other bitter constituents, nimbin (yield 0.1%) and nimbinin (yield 0.01%) have been obtained. The presence of gedunin, meldenin, desacetylgedunin, salannin, azadirone, epoxyazadiradione and a new minor product, vepinin (0.15%) is also reported in the oil. The seeds contain six new tetranortriterpenoids, viz, 1-methoxy-1, 2-dihydroepoxy azadirone, 1β, 2β-diepoxyazadiradione, 7-acetylneotrichilenone, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylepoxyazadiradione and 7-desacetyl-7-benzoylgedunin. The oil has also been shown to contain 0.47% of sulphur.
Azadirachtin (Morgan et al, 1973; Garg et al, 1984; Bilton et al, 1987; Kraus et al, 1987), shown to be a most potent insect anti feedant, was isolated from neem in 1968. Other compounds like salannin, 3-deacetylsalannin, salannol and salnnol acetate, isolated from the oil also show anti feedant activity.

Nimbidin has also been shown to inhibit growth of fungi. It has been used in preparation for external application in skin disorders. It also possesses anti arthritic, anti inflammatory and significant anti ulcer activity (Pillai et al, 1984).

1.6.1 Antifertility activity of Neem oil.

In addition to its many applications in treatment of various ailments, neem oil has also been shown to have antifertility properties. Sharma and Saxena (1959a,b) demonstrated that two derivatives of the neem oil- Sodium nimbinate and sodium nimbidinate had weak spermicidal action in vitro. The oil was also reported to have antiflagellal action (Chopra et al, 1982). Neem oil in its natural form was tested for its spermicidal activity in vitro and in vivo (Sinha et al, 1984a). Undiluted neem oil was found to possess strong spermicidal action (within 30 seconds) against monkey and human spermatozoa in vitro. In vivo when used intravaginally, a dose of 20ul in rats and one ml in monkeys and human subjects before sexual intercourse was found to be 100% effective in preventing pregnancy. The oil did not reveal any side effects as shown by histopathological studies.

It has also been reported that single or multiple intravaginal application of neem during pre-, peri- and post- implantation period could prevent pregnancy in rats (Sinha et al, 1984b). Subsequent to withdrawal of neem oil application for 30 days, there was 30% restoration of fertility. Histopathologically, the uterus, cervix and ovaries of the experimental animals were normal and the pups born after sub-contraceptive doses showed no abnormality.
Similar work has been done by other groups (Tewari et al, 1986; Lal et al, 1986) to study the post coital antifertility of neem oil. It was shown that neem oil when administered subcutaneously at different concentrations ranging from 0.05 ml-0.3 ml per rat per day for five or seven days after coitus showed significant antifertility activity ranging from 25-100 %. However, the oral route was not found to be as effective as no significant activity could be found on a 5-7 day regimen at much higher doses ranging from 2.5-5.0 ml per kg per day. In other studies (Khare et al, 1984; Lal et al, 1986) neem oil administered orally in a range of 2-6 ml per kg on a 10 day regimen post coitum showed significant antifertility action (20-40 %).

Recently, Riar et al (1990), reported that a volatile, odorous fraction of neem oil coded as NIM-76 obtained by steam distillation showed in vitro spermicidal activity at a concentration of 0.25 mg per ml for rat and 25 ml per ml for human spermatozoa. The spermicidal activity was not altered in the presence of vaginal or cervical mucus.

Very little work has been done to elucidate the mechanism of antifertility action of neem oil. One of the possible mechanisms suggested for the post-coital antifertility action following intra vaginal application (Riar et al, 1988) was the anti-estrogenic effect exerted by the oil around the time of implantation. However, other studies have ruled out this possibility (Prakash et al 1988), showing that neem oil did not possess any estrogenic, anti estrogenic or progestational activity. These workers have suggested that a subcutaneous dose of 0.3 ml\rat causes a severe damage to the uterine histological structures, making the uterine environment unsuitable for implantation.
Apart from the antifertility action of the oil, there are also reports of antifertility action of ethanolic and aqueous extract of neem. One ml aqueous extract of leaves of *Azadirachta indica* given orally to male mice for 30 days exhibited significant antifertility activity without inhibiting spermatogenesis. The block in fertility was reversible after 45 days of stopping the treatment (Sadre et al., 1980). On the other hand ethanolic extract of the seeds of neem did not show any significant activity in rats.

1.6.2 **Immunomodulatory properties of *Azadirachta indica***

In the last few years there has been a tremendous surge of interest in plants with immunomodulatory activity (Labadie et al., 1989; Patwardhan et al., 1990; Wagner et al. 1990). Immunomodulation has been described as any procedure which can alter the immune system of an organism by interfering with its function; if it results in an enhancement of immune reactions, it is termed as immunostimulation and primarily implies stimulation of function and efficiency of granulocytes, macrophages, complement, certain T-lymphocytes and different effector mechanisms. Immunosuppression implies mainly reduced resistance against infections and stress. The experimental field of research dealing with immunomodulatory activities in plants has been termed as immunopharmacognosy (Labadie *et al.*, 1989). Since immunomodulatory activity maybe expressed by different arms of the immune system, a battery of assays (Patwardhan *et al.*, 1990) are included for screening.

*Azadirachta indica* (neem) has been one of the few plants whose immunomodulatory activities have received a lot of attention, mainly due to its antimicrobial, antiinflammatory and antipyretic effect (Okapanyi and Ezeukwu, 1981). In addition, polysaccharides present in aqueous extract of stem bark have
been shown to possess antitumor, interferon inducing (Fujiwara et al, 1982; Teruma Corp. patent 1985) and anti-inflammatory activities (Teruma Corp patent, 1983). The immunomodulatory activity of *Azadirachta indica* stem bark have been studied in detail (Labadie et al, 1989). Dose dependent inhibitory effect was established on both pathways of complement activation as well as on the production of oxygen radical by activated PMN leukocytes. The inhibitory effect on classical pathway is much more pronounced than an alternative pathway. The aqueous extract was also shown to increase production of MIF, a lymphokine which in vivo attaches monocytes and macrophages to their site of action. The anti-inflammatory and anti-rheumatic effects of decoctions of *Azadirachta indica* bark have been correlated with in vitro inhibition of complement activation and superoxide anion production by PMN leukocytes. On the other hand the stimulatory property of aqueous extract on MIF production might be the underlying factor in generating stimulating and skin healing properties of the *Azadirachta indica* bark preparations in traditional medicine. From this lab, there is a recent study showing the immunomodulatory properties of neem oil following intraperitoneal administration in mice (Upadhyay et al, 1992). Neem oil was shown to enhance MHC-II expression and phagocytic activity of peritoneal macrophages. Significant gamma interferon production was also seen. The study indicated that neem oil acts as a non specific immunostimulant and that it Activate the cellular immune mechanisms to elicit an enhanced response to subsequent mitogenic or antigenic challenge.