INTRODUCTION
CHAPTER I
INTRODUCTION

The burgeoning world population and the associated risk of damage both to the planet and human health have led to calls for increased availability of family planning. According to the United States global population demographic estimates and projections, the world’s population will exceed 6 billion for the first time. Of this, some 80 percent will be living in developing countries (United Nations Population Fund, 1999). Recent data in the Times of India (2000) indicate that the world population crossed 6 billion mark with India all set to touch 1 billion mark. Asia has the highest population in all regions with India and China accounting for more than 40 per cent of the world population. Asia’s population numbers are almost 3.6 billion and currently has an average annual growth rates of 1.4 per cent. Population density is also the greatest in Asia, with more than 108 persons per square kilometer.

To stabilize the growing population, government should come out with a strong population policy and appropriate reproductive health care measures. As coercive methods of population planning have failed in the past, the new policy should make men and women equal partners in decisions regarding reproductive health issues without regarding the reproductive rights of the people. Whereas women bear most of the burden of reproductive ill health men’s involvement and cooperation is fundamental in the quest for improved reproductive health for both. As a result of increased understanding of a complex nature of reproductive health and gender dynamics, male reproductive health,
their active participation and sharing of responsibility for women’s reproductive health, have assumed a new reality in the 1990’s. The 1994 Cairo International Conference on Population and Development affirmed the need for ensuring male participation and responsibility in reproductive health by identifying the strategies that create an enabling environment (WHO, 1998; Wang, 1999).

The large number of unwanted pregnancies worldwide, and especially in countries where contraceptives are readily available is an indication that new and efficient contraceptive methods are needed. At the same time, high rates of discontinuation of existing methods and complaints about discomforting side effects are indications that contraceptive methods need to be made more acceptable.

It is now generally accepted that the currently available methods of fertility regulation are inadequate to meet the varied and changing personal needs of couples at different times in their reproductive lives and in the widely different geographical, cultural, religious and service settings that exist around the world. Contraception options for men are extremely limited. The ideal contraceptive method should be safe, effective and reversible and should not have an effect on libido. For biological reasons, women have to carry all the burden and risk associated with pregnancy and child birth. This however, does not imply that they should also carry most of the burden of fertility regulation. A sustained research effort is needed, if men are to have broader contraceptive choice to enable them to share effectively the responsibility for fertility regulation (Waites, 1993).

There are four main events in the reproductive process ovulation, production and
maturation of sperm, meeting of the ovum and sperm and implantation of the fertilized ovum that can be targeted in contraceptive approaches. New frontiers now opening up in science can yield novel ways of exploiting the other events in the reproductive process. The new opportunities can offer women and men a broader choice of better state-of-the-art contraceptives.

MALE CONTRACEPTIVE METHODS

NON-HORMONAL METHODS

Traditional Methods

Celibacy and Castration are the only completely reliable forms of contraception but neither is acceptable or practical for married couples apart from specific socio-cultural circumstances (e.g. religious orders, lactational taboos or social policy of delayed marriage).

Coitus interruptus and Periodical Abstinence

These are considered to be the common, easy method of male contraception. Coitus interruptus is one of the oldest methods of birth control practiced today and used by nearly 40 million couples for family planning (United Nations, 1989). However, the possible adverse psychological effects followed by a high failure rate are often associated. In certain men, the psychologic pressure of coitus interruptus may result in impotence or premature ejaculation and the woman may fail to respond fully. While reasonably effective for experienced users this method is demanding and has a correspondingly high
failure rate in practice (Trussell and Kost, 1987).

Condoms

Condoms are thin latex sheaths that fit over the erect penis and prevent semen from entering the vagina (natural membrane condoms, which protect against pregnancy but do not prevent HIV transmission are also available in some parts of the world). As with other barrier methods, the effectiveness of the condom depends on the experience of the user and the consistency of use. Typical failure rates among condom uses are approximately 12% in the first year of use. Condoms do not completely protect against sexually transmitted diseases (STDs), however areas of the skin not covered may be infectious or vulnerable to infection. Furthermore, not all condoms are of uniform high quality. Condoms are primarily, a temporary contraceptive method and the condoms have limited shelf life, particularly in tropical countries. Moreover, the acceptance of condoms varies with personal attitude. These are used by about 45 million couples for contraception and globally usage is increasing for prevention of STDs particularly HIV (Laskin et al., 1990). The major limitation of condoms is their relatively high failure rates (Trussell and Kost, 1987).

Voluntary Sterilization

Voluntary sterilization in the most widely used family planning method in the world and is of the most effective one. It is also one of the most economical means of terminating child bearing sterilization has the following benefits. It is permanent, highly
effective, and relatively safe and does not require continuing conscious involvement on the part of the user. While both male and female sterilization procedures are highly effective. Vasectomy is simple, safer and usually less expensive.

It is important that programmes offering voluntary sterilization include thorough counselling that emphasizes that surgical procedures are appropriate only for those who do not want any more children and that provides information about the alternative contraceptives available. Although sterilization techniques exist for both men and women they are not readily available, are very expensive, have low success rates and expose patients to unnecessary surgical risks.

**Male Sterilization**

Voluntary male sterilization - vasectomy is simpler, safer and usually less expensive than female sterilization. It is relied upon by over 40 million couples for family planning (Liskin, 1992). The vasectomy procedure involves minor out patient surgery done under local anaesthesia in which one or two small incisions are made in the scrotum and vas deferentia are cut and tied or otherwise occluded to prevent passage of the sperm. It is highly effective with a failure rate of 0.1-0.5% in the first year. Method failure is generally caused by spontaneous recanalization (reconnection) of the vas occlusion of the wrong structure during surgery or rarely failure to detect a congenital duplication of the vas.

After vasectomy the pressure in the lumen of the epididymis and proximal vas deferens may be increased and their internal environment of them may be changed.
Spermatogonia stored in the epididymis and proximal vas deferens may be gradually degenerated (Silber, 1978; Hong, 1989). The recovery of the sperm morphology after vasectomy reversal is not good (Pelfrey, 1982; Urry et al., 1990). So frequency rate is only 50-58% which may be related to formation of anti-sperm antibodies in serum (Hargreave, 1992). Changes of sperm parameters were also found in proximal vas deferens from vasectomized men (Wen, et al., 1999).

It is reported that after vasectomy the epididymal duct and proximal vas deferens may be distended (Silber, 1978) and there are more degenerated spermatozoa in the lumen of the cauda epididymis than that in the caput epididymis (Hang, 1989). Pelfrey et al. (1982) reported that after vasectomy one of the obvious changes in sperm morphology in the epididymis and proximal vas deferens was degradation of the sperm tail (Wen et al., 1999).

The safety of surgical vasectomy has been carefully monitored by the task force in the interest of the reproductive health of men. A major collaborative study allayed concerns that vasectomy might be associated with increased risk of cardiovascular ill health Tang et al., 1988; Petitti, 1986). Some studies suggest that vasectomy may predispose to prostate cancer (Guess, 1990). In addition an association between vasectomy and testicular cancer was suggested in some hospital based studies (Cale et al., 1990; McDonald, 1990). While these conclusions are encouraging, the task force still continue to monitor the safety of vasectomy.
No Scalpel Vasectomy

Although vasectomy is commonly performed using one or two small incisions in the scrotum, a 'no scalpel' technique developed in China is now widely used there and in several other countries. The no-scalpel technique is almost bloodless and appears to reduce the incidence of complications from hematoma. After a local anesthetic is injected, a specially designed ring forceps encircles and firmly secures the vas without penetrating the skin. A sharp tipped dissecting forceps is then used to puncture the skin and vas sheath and stretch a small opening in the scrotum. The vas is lifted out and occluded as with other vasectomy techniques. The same midline puncture site is used to occlude the other vas. No suture is needed to close the small wound which is covered with a small bandage.

Non surgical vas occlusion technique under evaluation includes percutaneous injection of liquid sclerosant (Liskin, 1992) or of polymers that harden into occluding plugs that may be removed later to restore fertility (Zhao, 1990).

The new sterilization technique developed in the Netherlands involves the injection of liquid silicone to block the two ducts vasa deferentia that carry sperm from the testes. The liquid silicone contains a hardener which enables it to set quickly and form a tight seal or plug within the vas deferens - thereby blocking the passage of sperm from the testis. The plug can stay in place indefinitely or be removed by a minor incision. If the injection pressure is too low, the liquid may not be in contact with interior wall of the duct before the plug forms - leaving gaps through which sperm can pass. However, further findings suggest that a slightly larger volume of silicone may be needed and
possibly a greater injection pressure. Other possible adaptations could include reducing the viscosity of the silicone to make it more fluid and quicker to disperse before setting.

Encouraged by Chinese success with the percutaneous injection of polyurethane elastomer to form plugs for intravascular occlusion (Zhao, 1990; Chen et al., 1992), collaborative study investigators have been initiated by the task force to explore the efficacy of the method when silicone is used as occluding material. Preliminary observations suggest that the method is effective but that the disappearance rate of sperm from the ejaculate is unaccountably slower than in conventional vasectomy (Zhao et al., 1992).

Two long term follow up studies were carried out in China on the efficacy and reversibility of two other methods of vas occlusion. Also a 10 center study was carried out to compare the effectiveness of reversal of three methods of vasectomy using microsurgery: no scalpel vasectomy (Li et al., 1991); vas occlusion with methylcyanoacrylate (MCA) and vas occlusion with medical grade polyurethane (MPU) plugs (Zhao, 1990). The reversal was found to be easiest in no scalpel group and most difficult in MCA group as a result of morphological changes induced in the vas deferens (WHO, 1998). Moreover the WHO (1998) identified vas occlusion a high priority leads among the male contraceptive technologies.

**Post Testicular Contraception**

The post-testicular or epididymal approach has the benefits of (1) almost immediate effectiveness; (2) ready reversibility and (3) avoidance of psychological or
endocrine impairment of libido. As sperm are matured and stored in the epididymis, under the influence of epididymal secretions (Cooper, 1986; Cooper and Yeung, 1999) contraceptive agents could influence spermatozoa indirectly, through disruption of epididymal epithelial cell function or act on them directly. The notion that contraception could be based on action in the epididymis is supported by several cases of natural infertility in domestic species (Cooper, 1992) and a knock out mouse model in which failure of epididymal development is associated with infertility (Sonnenberg-Riethmacher et al., 1996). The induction of infertility in males of several species through epididymal interference is more difficult to achieve by reduction of the amounts of epididymal secretions e.g. α-glucosidase, L-carnitine) or immunological interference with secreted proteins than by direct actions of drugs on sperm functions (e.g. inhibition of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) by chloro compounds). The latter approach holds promise for mankind as human sperm are susceptible to glycolytic inhibition. Future contraceptive developments may arise from production of targeted inhibitors, research on the displacement of sperm proteins in the epididymis and interference with sperm plasma membrane ion channels. From the data obtained so far from animal models, the induction of infertility in males of several species has been achieved by direct action of drugs on sperm function (glycolytic inhibition) rather than indirect actions mediated by modulating epididymal secretions (glycosidase, carnitine, sperm coating proteins). The indirect method has brought little success because epididymal secretions are not produced at limiting rates but accumulated at high concentrations within the epididymal canal by normal epididymal functioning that ensures
that all spermatozoa are affected by its secretions. Epididymal concentrations may have to be reduced enormously for an effect of their withdrawal to be observed and this may not be achievable rapidly or at all. The action of ornidazole and other pro drugs in inducing rapid, reversible infertility in males is currently an attractive post-testicular approach and is due to production of (S)-3-chloroacetaldehyde, a known inhibitor of sperm specific isoenzyme (GAPDH) and possibly the displacement of a protein involved in other aspect of fertilization. Further research with these compounds should combine these features with directed accumulation of agents around the sperm in the epididymis.

There are three major sites of action of an epididymal antifertility agent: (1) On peritubular muscle (hastening sperm transport leading to ejaculation of young, immature spermatozoa); (2) On the epithelium (altering the composition of epididymal fluid necessary for maturation and storage) and (3) On the spermatozoa (attacking their characteristic enzymes) (Cooper, 1992).

1. Action on peritubular muscle activity: By removing sympathetic innervation from the distal epididymis, normal sperm transport through the epididymis is affected (Ricker, 1996) and azoospermia can result as sperm accumulate in the epididymis. However this is a form of chemical vasectomy that suffers all problems associated with the surgical technique. Research on shortening sperm transit times remain to be done.

2. Action on epithelium: By inhibiting epididymal α glucosidase, the neutral form of glucosidase is secreted by the human epididymis (Cooper, 1990) and is found at a microvillous localization, also found in the rat (Yeung, 1990). Glucosidase
may be involved in the storage of sperm in the epididymis, but its effect on fertility is small. Hence, this approach does not look promising lead for a contraceptive.

By lowering the epididymal carnitine content: The high concentration of carnitine in epididymal fluid may reflect a high carnitine requirement of epididymal spermatozoa. As it has been claimed to be involved in the acquisition of sperm motility (Jeulin and Lewin, 1996), a reduction in the carnitine concentration in the epididymal lumen should affect sperm motility, and as a consequence fertility after natural mating. Carnitine is concentrated by the epididymal epithelium from the blood stream (Yeung, 1980). Total infertility was not induced in those animals whose epididymides were partially depleted of carnitine, so this approach to contraception did not look promising.

By immunological sequestration of specific proteins: Contraception is more likely when the epididymal secretions targeted are limiting and this may be the case for specific proteins that are taken up by spermatozoa as they mature or are stored. In rats active immunization against pre-albumin specific protein induces antibodies against the protein within days and reduced fertility within months (Cuasnicu et al., 1990; Ellerman et al., 1998). Immunization against pre albumin epididymal specific protein reduced in vivo fertility in weeks and epididymal sperm motility in weeks and zona binding was reduced in the immunized animals (Fournier-Delpech, 1985). As equivalent protein in men (Hayashi et al., 1996) is only loosely bound to the sperm surface it is unlikely that such immunization would achieve contraception in man (Kratzschmar et al., 1996).
hamster, antibodies formed after active immunization against epididymal specific protein P26H were found on the surface spermatozoa within the epididymal lumen (Berube and Sullivan, 1994). This method is capable of generating sufficient antibodies in the lumen to neutralise all proteins involved in fertilization. These promising results have led to clinical trials being planned for immunization against the human equivalent of this protein, P34H the amount of which on sperm is related to fertility of men (Boue et al., 1996; Boue and Sullivan, 1996).

**Interfering with sperm function:**

By interfering with sperm membrane ion channels: Adult homozygous c-ros tyrosine kinase knock-of-male mice by natural mating and the only phenotypic abnormality is in the epididymis that fails to develop an initial segment (Sonnenberg-Riethmacher et al., 1996). They are unique model to study the role of the epididymal epithelium in regulation of sperm function and present a possible point for contraceptive attack. Disrupting the production and function of the epididymal factors have contraceptive potential and is currently under study.

By inhibiting sperm glycolysis: Ornidazole is a nitromidazole compound that rapidly induces infertility in male rats without an effect on the testis (McClain and Downing, 1988) and has an effect on the motility of cauda epididymal spermatozoa. The rapid action implies post testicular effect but since no changes in epididymal secretions were observed a direct action on epididymal spermatozoa was concluded (Oberlander, 1994).
Preliminary studies have indicated that sperm motility and glycolytic enzymes are inhibited by these compounds.

By displacing sperm proteins: Contraception Associated Protein (CAP1) appears in epididymal fluid of infertile rats as ornidazole drug induced loss from the sperm, suggesting that this protein is of importance for fertility (Wagenfeld et al., 1998a). Further characterization of the protein and determination of its nucleotide sequence indicates an identity with a human proto oncogene (Wagenfeld et al., 1998b). The presence of a human analogue brings hope that contraceptive attacking this protein will have application in humans.

**Plants and plant products**

Plant products constitute approximately 25% of all the prescribed medicine even in the most advanced countries like USA. The development of safe, orally, reversible effective fertility regulating agents from plants for human beings is not a new idea. For centuries, virtually every ancient culture has utilized plants as one form or another in an attempt to control population with the relative paucity of new leads for male contraception development. The future prospects lie in plants and their products as potential sources of drugs. It is conceivable that systemic analysis of ethno pharmacological information may yield promising leads in the search for new fertility regulating agents (Kong et al., 1986). Numerous plants and their products have been screened for their antifertility effects in laboratory animals and man. But their use as antifertility agents are hindered due to their
toxicity on other reproductive and non reproductive tissues (Waites, 1988).

The antifertility effect of *Ocimum santum* L. in male mice by feeding the leaves along with normal diet was shown by Kasinathan et al. (1972). The treated males fail to fertilize normal female mice of proven fertility although copulation plug was found in some females. The extract of *Azadirachta indica* manifested antifertility effect in male mice (Deshpande et al., 1980). The effect of plumabagin on spermatogenesis and accessory reproductive organs of rat (Santhakumari et al., 1980) and *Puevaria tuberosa* were also investigated (Daftari et al., 1981).

Nagarajan et al (1982) have tested numerous indigenous plants having potential spermicidal activity in human beings and in animals. The green flower extract of *Malvaviscus conzatti* exhibited antifertility effects in male albino mice (Verma et al., 1980). The crude seed extract of *Arbus precatorius* Linn, caused testicular lesion (Baijal et al., 1981). Rao (1987) also reported antifertility effect of aqueous and alcoholic extract of these seeds on fertility in males. Chinoy and Geetha Ranga (1983) have reported that leaf extract of *Vinca rosea* L., manifested 100% antifertility and strong antiandrogenic effects in adult male albino rats. *Carica papaya* have also been screened for their antifertility activity (Chinoy et al., 1994; Lohiya et al., 1992). Rao (1988, 1989) has reported a definite antifertility effect of the alcoholic extract of *Terminalia bellirica* fruit and *solanum xanthocarpum* seeds in male rats. Rao et al. (1997a) reported the antifertility effects of the pricarp of fruit extract of *Balanites roxburghii* in male mice. Rao et al. (1997b) further studied the contraceptive effect and spermicidal action of *Phyllanthus amarus* in rodents from our laboratory.
Following clinical trials conducted in China in the 1970s, gossypol was proposed as a drug for male contraceptive use. Although the interest in gossypol had stimulated much innovative research the decision was taken to discontinue work on gossypol as a potential contraceptive drug (Waites; 1988) and the basis of this decision was fully reviewed (Waites, et al., 1998). Trials conducted in Chinese volunteers established that gossypol treatment directly induced hypokaelamia, possibly by impairment of kidney function (Liu et al., 1988; Michael, 1998). Finally this evidence of animal and human toxicity together with the high incidence of irreversible testicular damage led to recommend that no further clinical studies should be undertaken with gossypol as an antifertility agent.

A multiglycoside extract of plant *Tripterygium wilfordii*, long used in Chinese traditional medicine for the treatment psoriasis was shown to cause reduction in sperm motility and concentration in male patients (Qian, 1987). A series of diterpene epoxides from extracts of root bark of the plant were isolated. Several were shown to be orally active in rats at exceedingly low doses. One triptolide, was found to induce complete infertility in male rats acting primarily on epididymal sperm with minimal effects of the testis (Lue, 1998). This is encouraging but the path for triptolide to become marketable contraceptive drug is long and expensive and would require the involvement of a pharmaceutical company (WHO, 1998).

**HORMONAL METHODS**

These are the most advanced type of the contraceptive methods for men. The
suppression of sperm production by hormonal means has been a general research strategy of all agencies interested in male contraception. Various drugs either alone or in combination, have been tried (Waites. 1993; Cummings et al., 1994). The studies carried out in this area have focussed on three main approaches (a) the inhibition of spermatogenesis by the suppression of secretion of gonadotropins, both LH and FSH or FSH alone; (b) recovery of circulating androgen to physiological levels without re-stimulation of spermatogenesis and (c) the assessment of the functional capacity of residual sperm.

Research has focussed on the selection of a variety of hormonal drugs that can effectively suppress pituitary hormone secretion. A number of studies in animals and men have shown that the administration of androgen alone, combination of gonadotropin-releasing hormone (GnRH) and androgen, and progestogen and androgen combinations, can suppress gonadotrophin secretion and spermatogenesis either completely to azoospermia or to sufficiently low level of oligozoospermia to render the treated individuals infertile. Furthermore discontinuation of treatment leads to full recovery of gonadotrophin secretion and spermatogenesis and return of fertility.

Effective suppression of spermatogenesis requires a profound suppression of both gonadotropic hormones and of intratesticular testosterone concentrations. Weekly injections of testosterone enanthate (TE) a safe androgen in widespread use in clinical andrology was the regimen chosen to establish the degree of sperm suppression needed for contraceptive efficacy. Administration of testosterone enanthate 200 mg/week induced azoospermia in about 60% and severe oligospermia in almost all remaining men. TE was
not envisaged as abusable contraceptive drug.

A number of approaches are being investigated to maintain the same suppression/replacement levels of testosterone by less frequent administration of the hormone. These include biodegradable microcapsule loaded with testosterone, testosterone pellets and long acting testosterone esters. Consequently, the encouraging results of these studies have stimulated interest in new long-acting drugs. For example novel formulations of testosterone esters, testosterone undecanoate (Zhang et al., 1997) or by testosterone buciclate (Behre et al., 1995; Behre and Nieschlag, 1997). These may be administered alone or combined with progestagens such as depot medroxy progesterone acetate (DMPA) (WHO, 1993) and levonorgestrel or desogestrel (Wu, 1997) or with GnRH analogues (Behre and Nieschlag, 1997). Although testosterone alone is effective in suppressing spermatogenesis to a contraceptive level, a much higher than normal physiological concentration in circulation is needed in order to achieve this effect. The use of a progestagen to suppress spermatogenesis would enable the replacement androgen to be provided at a dose within the physiological range. It is conceivable that combination of long acting progestogens with long acting androgens that are being developed can form basis of a 'male contraceptive pill'.

ESTROGENS

All estrogens are related to the parent structure estrone (18 carbons) and possess an aromatic A ring and certain oxygenated substituents at C-3 and C-17. The most biologically active estrogen produced in the body is estradiol. Oxidation of the hydroxyl
group at C-17 of estradiol gives rise to estrone which is 30% to 70% less active than estradiol. Addition of a hydroxyl group at C-16 of estradiol yields estriol which is one-tenth as potent as estradiol from β to α orientation gives rise to 17α estradiol which is biologically inactive.

A number of potent estrogens have been synthesized and are used orally or parenterally. Structural modification of estradiol molecule by insertion of an ethinyl group at C-17 results in formation of ethinyl estradiol, a very potent estrogen with high oral activity. Long acting, oil soluble estrogenic preparations used therapeutically are synthesized by esterification of estradiol at C-3 or C-17 with fatty acids. In general the higher the molecular weight of estradiol derivative, the more pronounced is the biologic activity of the compound.

Estrogens are potent inhibitory of gonadotrophin release in the male, inhibiting spermatogenesis and reducing testosterone secretion. Also they tend to reduce libido. The inhibitory effect of estrogen on testicular steroidogenesis is well demonstrated (Dufau et al., 1978). Rao and Chinoy (1984) and Rao et al. (1993; 1994) showed its effects on male reproductive system using diethyl stilbestrol (DES) and androgen combination. It has been reported that the estrogen treatment to mice manifested epididymal maturational changes in spermatozoa as a result of androgen deprived effect (Rao and Mathur, 1987). Estrogenic compounds have been used as a potent antispermatic and antifertility agents in males (Rao and Chinoy, 1983). The potential contraceptive efficacy of a combination of testosterone and estradiol 17β or DES has been suggested by Ewing et al. (1977) and Rao et al. (1993; 1994). The results suggested that the rats rendered
azoospermia and were sexually active but predictably infertile. Similar combination administered via silastic implants were also found to be effective (Ewing et al., 1979; Robaire et al., 1979). Estradiol benzoate (E$_2$B) is which is formed by esterification of estradiol with benzoic acid showed androgen antagonistic and antifertility effects in rats (Chinoy and Rao, 1982; Rao and Chinoy, 1993; 1984). It was concluded that E$_2$B interfered with the microenvironment of the epididymis and had androgen deprivation effect on the target organs. Present studies support the contention that non steroidal antiestrogens can modulate estradiol induced epididymal response during the post-natal period of male rat (Dhar et al., 1997). A study by Yang et al. (1997) has elucidated the mechanism of action of estrogens and antiestrogens. Oestrogens bind to the oestrogen receptor, inducing a conformational change that leads to activation of gene transcription through specific estrogen response (El Hajj Fuleihan, 1997). However estrogens with addition of androgens are undesirable for the contraceptive use in the male because of the feminizing symptoms such as gynecomastia and other adverse effects.

**PROGESTINS**

Progestins have been used as antifertility agents in the male due to their antispermatogenic potential (Bennet, 1974). They have been classified into the following categories.

**Progesterone and its esters**

Progesterone has been reported to be a weak antispermatogenic in many species
(Ericsson et al., 1964; Ericsson and Dutt, 1965). Because of their weak antigonadotrophic effect (Bennet, 1974), these compounds poorly inhibit the spermatogenesis and this activity may be further negated by their ability to act as substrate for testosterone synthesis.

**Progestagens**

These synthetic progestins include 19-nor-testosterone derivatives. The 17-acetoxy derivatives of progesterone are generally poor antispermatogenic agents. However, the results are variable and confusing. Medoxyprogesterone acetate was thought to inhibit spermatogenesis without altering libido, in humans but the results were not forthcoming in the second trials (Patanelli, 1985). Megesterol acetate (17-acetoxy, 6 methyl \( \Delta^6 \)-progesterone) did not exert antispermatogenic effect in rats (Karkun and Kar, 1965).

**Antiandrogenic progestins**

The antispermatogenic properties of progestagens are exceedingly variable and dependent on the amount and type of progestagen administered. Moreover, adverse side effects especially related to hepatic dysfunction were also observed (Bruce et al., 1978).

In order to produce the required degree of azoospermia or oligospermia higher amounts of progestins are required (Bajaj and Madan, 1983). At sufficient dose administration, induced severe oligospermia or azoospermia also result in loss of libido and potency as well as increased nipple pain. Hence, the application of simultaneous androgen therapy would be preferred (Roy, 1994)
normal men and they demonstrated suppression of spermatogenesis. Similarly, azoospermia condition was also observed by testosterone administration through sustained release from capsules in rabbit (Ewing et al., 1973; Reddy and Prasad, 1973).

Long acting esters of testosterone induced suppression of spermatogenesis but the frequency of administration and dosage were critical in establishing and maintaining spermatogenic suppression (Cunningham et al., 1978; Steinberger et al., 1978; Swerdloff et al., 1978). The most widely studied preparation of long acting ester is testosterone enanthate (TE) and it has been found to cause a marked suppression of spermatogenesis. A long term effect of androgen used as a fertility regulating device was investigated by Paulsen (1985) and Robaire et al. (1984). An indication that kinetics closer to zero release order may be more effective and is provided by studies using 19-nor testosterone ester with longer half life than testosterone enanthate (Knuth et al., 1985; Behre et al., 1990). The testicular spermatogenesis was affected and the epididymal sperm motility and sperm concentration showed a decline after biweekly injection of TE for 30 and 60 days (Rao et al., 1996).

A single intramuscular injection of testosterone trans-4-butyl-cyclohexylcarboxylate (20 AET-1) administered to castrate male cynomologus monkeys (Weinbauer et al., 1986) could maintain the serum testosterone levels in physiological range upto 4 months and appears suitable for human application (Rajalakshmi, 1994).

Numerous efforts have been made by combining testosterone esters with other compounds such as progestagens and LH-RH analogs for use in male contraception (WHO, 1994).
ANTIANDROGENS

Most estrogens and progestogens are antiandrogens. Any gonadotrophic substance too can be considered as antiandrogen which reduced stimulation of Leydig cell testosterone synthesis. That means antiandrogens are compounds which prevent the expression of biological activity of androgens at target sites. These substances inhibit or block the androgenic effect of testosterone at central and peripheral receptors.

It means the second type of antiandrogen act by competing with androgens for their receptors in peripheral target tissue so that androgenic response to a particular concentration of testosterone in the circulation diminished. Such compounds are rare, though cyproterone is one. It exerts negative feed back on hypothalamus (Brotherton and Harcus, 1973). But Morse et al. (1973) suggested that cyproterone administration directly inhibits testosterone secretion and effects libido and potency. Accordingly these antiandrogens cannot be used for fertility regulation (Neumann et al., 1978).

Many other substances have both peripheral androgen antagonism and antigenadotropic properties. Cyproterone and cyproterone acetate (CPA belonging to a class of antiandrogens with additional progestational properties were considered suitable for fertility regulation. Due to their unfeasible nature co-supplementation of androgen is under trial in human beings (WHO, 1998).

ANDROGENS

Androgens for male contraception have been extensively investigated during the last few decades. Reddy and Rao (1972) used testosterone propionate 50 mg daily to
ANDROGEN-PROGESTIN COMBINATION

The lack of success in inhibiting male fertility by a single hormone has produced the suggestion that hormone combinations should be considered (Bain, 1980, WHO, 1994). In addition, the dose of both the androgen and the progestin administered in combination may be less than that of these compounds administered alone. A whole host of different gestogens were studied in combination with testosterone and its esters in a series of clinical trials sponsored by the population council.

Various hormonal regimens have been tested for suppression of spermatogenesis, sufficiently to act as an effective contraceptive (Knuth and Nieschlag, 1987; WHO, 1992; 1994).

Among the combination tested so far depot medroxy progesterone acetate (DMPA; 200 mg) and testosterone cypionate (250 mg) monthly seems to be promising combination (WHO, 1979). 200 mg of DMPA with 200 mg testosterone enanthate (TE) monthly was effective with incidence of low side effects. It was shown that a long acting ester of 19-nortestosterone (19NT) alone (Schurmeyer et al., 1984; Knuth et al., 1985) or combination with DMPA (Knuth and Nieschlag, 1987; WHO, 1993) was equally effective in producing sperm suppression like testosterone enanthate. Rao and Roy (1993) and Rao et al. (1995; 1996; 1998) demonstrated altered sperm function in rats administered with a combination of MPA + TE in rats. Testosterone esters like testosterone undecanoate (Zhang, 1997), testosterone buciclate (Behre et al., 1995; Behre and Nieschlag, 1997) may be administered alone or in combination with latest progestogens such as DMPA.
PROTEIN/PEPTIDE HORMONES

GnRH analogue

Clinical studies (Pavlou et al., 1991) and studies in non-human primates (Weinbauer et al., 1989) have shown that GnRH antagonists are more potent in the suppression of gonadotropin secretion and of sperm production than are GnRH agonists. Research on these compounds is well justified for their application in the treatment of cancers but the cost of synthesis of peptide hormones such as GnRH antagonists, is likely to remain too high for contraceptive use in developing countries.

GnRH agonists

With the synthesis of analogues of the decapeptide GnRH, major advances have been made in understanding the factors regulating male reproductive functions and the possibilities of intervention leading to arrest of fertility. Potent agonist of GnRH results in desensitization of pituitary and consequent inhibition of gonadotropin secretion. Simultaneously agonists exert a direct action on the testis by inhibiting LH action on Leydig cells.

The paradoxical antifertility effects induced by chronic administration of GnRH agonists to laboratory animals and the human (Belanger et al., 1980; Sandow et al., 1978; Smith et al., 1979) led to extensive studies using primates to test their potential as male contraceptives. A review of the published work shows that azoospermia could not be induced in monkeys by daily administration of a number of GnRH agonists. Daily administration of the potent GnRH agonist, LHRH to adult rhesus monkeys did not result
in consistent reduction in sperm count (Sundaram et al., 1984). While with manipulation of the doses, duration and mode of administration of the agonist and the androgen one may successfully induce azoospermia in limited clinical trials, such regimens would be only of academic in the context of development of a male contraceptive.

**GnRH antagonists**

In view of the inability GnRH agonist to induce uniform azoospermia, and the need for androgen substitution which results in stimulation of spermatogenesis, attention was focused on the use of GnRH antagonists for male fertility regulation. GnRH antagonists act directly on the pituitary GnRH receptors (Heber et al., 1982) and inhibit both pituitary and testicular functions (Adams et al., 1986; Akhtar et al., 1985). However some of the GnRH antagonists assessed for their antifertility effects also showed adverse side effects mainly related to their propensity for histamine release (Karten and Rivier, 1986). Research efforts in this field received impetus with the synthesis of potent GnRH antagonists without associated side effects. However, the use of these antagonists necessitated androgen substitution to ameliorate the antagonists induced androgen deficiency.

A close scrutiny of the data from various studies raises some concern in using GnRH antagonists for male fertility regulation. These are (1) In all studies, even with androgen supplementation, a significant decrease in weight loss was noticed; (2) In a clinical study conducted by Pavlou (Pavlou et al., 1991) men with higher body weight
were not as responsive to induction of azoospermia by the antagonist as men of lower body weight. The heavier subject required higher dose of antagonist; (3) In men with prostatic cancer and on treatment with the GnRH analogue histological examination of testis showed marked tubular thickening and fibrosis. These observations raised the possibility of the irreversible nature of such treatment (Smith and Urry, 1985). WHO task force has initiated studies on reversibility of testicular functions in bonnet monkeys and long-term GnRH agonist treatment. Similar studies using antagonists are needed to assess if such long-term treatments are reversible.

**Immunological approach**

**Vaccines**: The immunological approaches to interrupt male fertility by targeting sperm surface epitopes or the hormones regulatory spermatogenesis have long been of interest. Sperm are autoimmunogenic as they first appear within the immunologically protected adluminal compartment of the seminiferous tubules long after establishment of immune self tolerance. Steps required to develop a sperm vaccine include the identification of suitable surface expressed epitopes that are sperm specific, adequately immunogenic and involved in fertilization (Aitken et al., 1992). Unresolved problems remain over the large antigenic burden in the male genital tract requiring virtually complete blockade, the variability of individual immune responses, difficulties identifying an effect adjuvant safe for human use, the restricted access of antibodies into the seminiferous tubules and epididymis and the risk of orchitis or other immune complex disease. Nevertheless the improved understanding of molecular physiology of sperm function and fertilization
provides new defined molecular targets while improved biotechnology can supply target epitopes in unprecedented abundance greatly improving the technical feasibility of immunological interruption of male fertility.

**Cytotoxins**: Numerous chemical and physical cytotoxins including alkylating drugs (Meistrich et al., 1982), heat (Kandeel and Swerdloff, 1988) and ionizing irradiation (Meistrich and Beek, 1990) readily and reversibly abolish spermatogenesis. This is due to high susceptibility of the rapidly proliferating germinal epithelium to direct damage of cellular and DNA replication. The mutagenic risks of directly disrupting DNA synthesis are however undesirable for a reversible male contraceptive and therefore chemical methods to regulate male fertility aim at inhibiting sperm function rather than spermatogenesis. Therapeutic drugs that impair male fertility may have potential for development as male contraceptive however surveillance of male reproductive side effects is incomplete and unsystemic (Vickery et al., 1986) Potential models include orally active spermicides concentrated in semen (Vickery et al., 1986), sulphasalazine which causes sulfonamide related reversible subfertility although the mechanism remains obscure (Giwercman and Shakkeback, 1986) and drugs that prevent ejaculation without affecting sexual potency or spermatogenesis (Hormonnaï et al., 1984; Kjaergaard et al., 1988), however none of these has yet been effectively developed (Puri and Van Look, 1994).

**SPERMATOGENESIS**

The mammalian vertebrates testes have been evolved dual functions of being both
exocrine and endocrine. Its primary function is to produce sperm, the male gametes. It also secretes the male sex hormones, the androgens, which regulate spermatogenesis, development and differentiation of accessory reproductive organs and synchronize their functional physiology. These two functions of the testis are shared by two specialized compartments of the testis. The seminiferous tubules, the avascular compartment of the testis produces sperm. The vascularized interstitial tissue containing the cells of Leydig produce androgens. The seminiferous tubules form about 70% to 90% of the total mass of the testis.

The epithelial lining of the seminiferous tubule is called the seminiferous epithelium. Seminiferous epithelium contains two types of cells. An interdependent mass of the proliferating cells, the germ cells. They produce sperm. The second type of the cells are the non-proliferating group of irregularly shaped sessile Sertoli cells. The Sertoli cells provide architectural support to the tubules and also a micro-environment for the germ cells undergoing the process of sperm formation. The Sertoli cells constitute about 25% of the cellular population of the seminiferous epithelium.

The process of formation of spermatozoa from germ cells is called spermatogenesis. Spermatogenesis takes place within the seminiferous tubule. Spermatogenesis is highly organized process. It is a dynamic continuous process. Spermatogenesis involves two types of cell divisions, the mitosis and the meiosis. It also includes the morphogenesis of spermatozoa called spermiogenesis. Spermatogenesis involves distinct phases during which the cytoplasmic and nuclear components of the germ cells undergo specific morphological and biochemical changes. The basic cell type
Figure 1: Spermatogenesis in mammals
which undergoes spermatogenesis is to become a spermatozoan called the spermatogonium. Spermatogonia divide by mitosis to produce spermatocytes. Spermatocytes divide by meiosis to produce a haploid cell type, the spermatid. Spermatid undergoes extensive remodelling to produce sperm. So spermatogenesis is a combination of regular synchronized complex process of cellular differentiations. This is a long drawn out process involving the production of distinct germ cell types which are arranged in regular concentric layers around the lumen of the seminiferous tubule and within it. This arrangement of germ cells is very distinct and specific. The earlier stages of germ cells are present in the outer layers nearer to the basement membrane of the tubule. The later stages of germ cells are present in the inner layers found towards the lumen of the seminiferous tubules.

Though only three cell types are identified in spermatogenesis, each of these cell type show several generations and forms. Spermatogonia are the earliest germ cell types and they are diploid. They constitute the basic type. They renew themselves many times before producing spermatocytes. Spermatocyte undergoes reduction division to produce haploid spermatids, which undergo a series of morphogenetic modelling (spermiogenesis) to produce sperm (Fig.1).

The nucleus of the spermatid is small and sphericle at the beginning of spermiogenesis. With the progress of spermiogenesis the nucleus enters an elongation phase. Initially it increases in size and then decreases. There is condensation of chromatin. During spermiation there is the formation of a structure called manchette. It is formed by the interlinking of cytoplasmic tubules. It establishes links with the nuclear envelope.
Figure 2: Hormonal regulation of steroidogenesis and spermatogenesis in the testis (Bolander, 1989; Carr and Bhackwell, 1993)
through a group of fibers. It forms a rigid cytoplasmic apparatus. It is involved in nuclear morphogenesis.

Four important events occur during the formation of spermatozoa: (a) formation of a locomotor apparatus; (b) condensation of chromatin; (c) inactivation of the genome, which helps in conserving the totipotency of the cell and (d) differentiation of structures which assist the spermatozoa in recognizing complementary molecules on the surface of the egg during fertilization. They may also help in penetration of egg investments.

Fully formed spermatozoa are held by Sertoli cells at their head and with their tail trailing into the lumen of the seminiferous tubules. They get released into the lumen and is called spermiation. The residual bodies left behind after spermiation get phagocytosed.

Hypothalamic factors, hypophyseal gonadotropins (Gns), androgens and a number of other factors regulate spermatogenesis (Fig 2).

**HORMONAL CONTROL OF SPERMATOGENESIS**

Spermatogenesis requires both FSH and very high levels of testosterone. The testosterone is supplied by the surrounding Leydig cells and is concentrated in the tubules by a special androgen binding proteins (ABP) produced by Sertoli cells that are induced by FSH. Luteinizing hormone (LH) stimulates testosterone synthesis by inducing the desmolase complex in the Leydig cells.

Low steroid levels release the hypothalamus and pituitary hormones from feedback inhibition. The resulting gonadotropin releasing hormone (GnRH) secretion stimulates LH release which in turn activates steroid synthesis in the Leydig cells.
the steroid levels have been restored feedback inhibition is reestablished. It appears that the Sertoli cells monitor spermatogenesis and release a hormone, inhibin, that specifically inhibits FSH secretion.

FSH stimulates spermatogenesis by inducing renewal of type A spermatogonia. LH induces Leydig cells to synthesize androgens. Androgens maintain spermatogenesis. FSH acts directly on the seminiferous tubular initiating mitotic divisions of spermatagonia. Once spermatogenesis has started, there is no need for continued presence of FSH to maintain spermatogenesis. FSH initiates a number of metabolic activities in the seminiferous tubule. FSH stimulates the enzyme adenyl cyclase which in turn induces the formation of an active protein, kinase. FSH is necessary for completion of morphogenesis during spermiogenesis. Any change in hormonal balance affects the events in the basal compartment of the Sertoli cells. The Gns and testosterone may act on Sertoli cells directly or indirectly. As an end product of hormonal (FSH) action, androgen binding protein (ABP) is produced by the Sertoli cells. They also produce inhibin protein, that controls FSH production. Thus Sertoli cells act to create a favorable microenvironment for spermatogenesis. LH and FSH synthesis and release are mainly regulated by the testicular hormones, viz., testosterone and inhibin respectively. Sertoli cells also can synthesise estrogens by using precursor steroids produced by the Leydig cells (Lamming, 1990).

**EPIDIDYMIS AND ITS FUNCTIONS**

The epididymis resting on the dorsolateral aspect of the testis, consists of the
ductuli efferents and the ductus epididymis, encapsulated by the tunica albuginea. Some 8-12 coiled ductuli efferents originate from the rete testis and constitute most of the caput epididymis (Mann and Lutwak-Mann, 1981). The ductuli efferents join the coiled ducts epididymis, which forms the corpus and cauda segments of the epididymis. The combined lengths of these ductular system measure 5-6 meters and they transmit the sperm and fluid from the testis to the vas deferens. Holstein (1975) recognized eight segments in the epididymis with a proximodistal decrease in the width of the lumen, with the narrowest portion at the transition of the corpus and cauda epididymis.

The epididymis epithelium consists of two cell types, e.g. principal cells and basal cells. Principal cells are high, columnar elements. The apical portion of the cell has many membrane bound vesicles, multivesicular bodies, lysosomes and stereocilia Cytoplasmic protrusions extend into the lumen

The histologic characteristics of the principal cells vary along the different segments of the epididymis. The basal cells are spherical and occur randomly between the more numerous principal cells. They do not extend towards the lumenal surface of the epithelium.

Varying amount of sperm, immature germ cells, as well as fragments of cells such as nuclei, organelles, or membranes are found in the ductus epididymis. Intermingled with such sperm constituents are epididymal epithelial cells in various stages of disintegration and large phagocytes containing spermatozoa or fragments of cells (Holstein, 1975; Knobil et al, 1988, Rao et al., 1988).
Functions

The epididymis performs several functions. Spermatozoa are transported from testis to the ejaculatory duct through the epididymis. Sperm undergo maturation and achieve motility and the capacity to fertilize during their passage through epididymis. The spermatozoa are stored in the cauda epididymis and vas deferens (Hafez, 1977). Depending on the frequency of ejaculation there is an equilibrium between the breakdown of aged spermatozoa and fullness of the epididymis. The epididymal epithelium has both secretory and absorptive functions under the strict control of prevailing androgens (Lamming, 1990; Rao and Shah, 1998). It secretes the complex epididymal plasma in which sperm are suspended and undergo maturation. The testicular fluid that transports the sperm from the seminiferous tubules is resorted in the efferent ducts and in the proximal portion of the epididymis. Products of the sperm breakdown are also resorted in the epididymis.

Sperm maturation in the epididymis involves morphological, physiologic and metabolic changes.

The most striking morphological change involves the cytoplasmic droplets a remnant of spermatid cytoplasm that migrates caudally from the neck of the spermatozoa to the end of the mid piece. This extent is accompanied by dehydration and ultrastructural changes in the droplets. The cytoplasmic droplet is distinguished by the presence of lysosomal enzymes that may be involved in the final maturation of the epididymal sperm. The metabolic activity of the sperm increases as they progress through the epididymis as indicated by an increase in the rate of fructolysis and in carinitine content, possibly aiding
in glycolysis and oxidation of lactate and acids. Maturational changes also involve an increased capacity for fertilization and development of directional motility (Orgelbin-Crist, 1984).

Intrinsic factors such as ageing are not sufficient for sperm maturation and have emphasized that the epididymal environment is needed for spermatozoa to develop fertilizing capacity. Biochemical changes in the epididymis may play a major role in the maturation of spermatozoa.

In the cauda epididymis, the main driving force for water movement is the active transport of sodium ions across the epithelium whereas in the caput and the corpus epididymus, chloride transport followed by passive movement of sodium ions is the probable driving force (Wong et al., 1978). The major secretory/synthetic products of the epididymis are glycerol phosphoryl choline (GPC), phospholipids, carbohydrates, sialic acids, proteins, enzymes, steroid binding proteins, carnitine, vitamins, metal ions, all of which are androgen dependent.

Epididymal plasma contains a large amount of GPC in several species (Hamilton, 1977). Recent studies (Hinton, 1980), have suggested that GPC, phosphocholine and inorganic phosphocholine are secreted in the caput epididymis of rat and during sperm maturation. Some amount of these compounds are lost along the epididymis.

Numerous histochemical, quantitative and autoradiographic studies have demonstrated a variety of carbohydrates and enzymes involved in the metabolism of the epididymis (Hamilton, 1977).

The epididymis has hydrolytic and oxidative enzymes which are androgen sensitive.
(Several papers from Prasad and his group; Chinoy, 1984 and several others). Sialic acid and sialomuco protein are secreted by the epididymis of a number of mammals including human being (Hinton, 1980). Sialo proteins are associated with changes in the membrane surface of maturing spermatozoa and development of the fertilizing capacity of sperm (Rajalakshmi, 1985).

VAS DEFERENS AND ITS FUNCTIONS

From each epididymis a duct, a vas deferens passes. The vas deferens is a continuation of the epididymal duct, beginning at the point where the ductus epididymis straightens and reverses its direction towards the inguinal canal. It is a tubular structure which serves as a passage for sperm transport during ejaculation. It also participates in absorptive and secretory functions. The vas deferens is generally considered to the mesonephric duct origin arising from the middle segment and this develops during gestation under the influence of testosterone. The vas deferens is surrounded by a thick muscular layer.

Morphologically, the vas deferens can be differentiated into two regions, the proximal vas deferens which is near to cauda and the distal vas deferens adjacent to accessory sex gland complex. Significant differences exist in the diameter of proximal and distal region (Chinoy, 1985). In mice, the vas deferens can be sub divided into three sections recently

(a) The proximal vas deferens, located primarily in the scrotum, is flattened due to distribution of logitodinal muscle layers but contains a tubular lumen which is
(b) The distal vas deferens in the inguinal region is circular in cross section due to the presence of thick longitudinal layers. The structural features of the epithelial cells differ from those in proximal vas deferens and

(c) The terminal region of the vas deferens lies in the abdominal pelvis and terminates at the point where it is joined by the duct of seminal vesicles to form short ejaculatory duct (Knobil et al., 1988; Lobo et al., 1997).

Histologically each region of the vas deferens can be differentiated from the other on the basis of characteristic epithelium, lamina propria, shape of lumen and the presence or absence of folds in epithelium. The epithelium is cuboidal and the lumens is highly distended. Four different cell types are recognised in the epithelium, namely the principal cells, pencil cells, mitochondria rich cells and basal cells. As one moves from proximal to distal segment of the vas deferens, the height of the epithelium increases because of the tall columnar principal cells, basal cell and halo cells still present, while clear cells are no longer seen. The lumen narrows and the distended appearance changes to one that is convoluted. There is a decrease in the content of spermatozoa as one progresses from the proximal to distal part of the vas deferens. There is a marked increase in the thickness of the smooth muscular layer surrounding this epithelium. The muscle coat is composed of three layers: an inner longitudinal layer, a middle oblique or circular layer and an outer longitudinal layer.

Elastic fibers are prominent in the lamina propria where they from two layers and they are also present among the smooth muscle cells of inner muscle layer.
In the distal ductus deferens, the basic part of the cells have an enormous accumulation of smooth endoplasmic reticulum (Flickinger, 1973; Wenstrom and Hamilton, 1984). In the terminal ductus deferens, Cooper and Hamilton (1977) identified nests of phagocytic cells, that phagocytose defective sperm.

The innervation of the vas deferens is extremely complex and has been reported to involve adrenergic, purinergic and peptidergic neurotransmission.

Blood supply to vas deferens by way of the internal liac, hypogastric and finally the internal spermatic arteries. The returning venous blood travels in the differential vein which returns along the ductus deferens and empties into the hypogastric vein (Knobil et al., 1988).

Functions

Flickinger (1973) suggested on the basic of structural features that the proximal vas deferens functions in the synthesis of protein/glycoproteins and in the absorption of material from the lumen. Further studies in our laboratory have revealed that both regions of the rodent vas deferens possess absorptive, synthetic and secretory activities (Chinoy, 1985; Rao et al., 1998).

The vas deferens is not a mere passage for sperm transport but it is an important organ contributing actively to the maintenance of sperm structure, maturation, survival and viability (Chinoy, 1985; Robaire and Hermo, 1988). It was reported that the proximal and distal vas deferens of albino rats possessed differential sensitivity to testosterone where the distal vas deferens had a higher threshold requirement of testosterone for the
maintenance of its structural and biochemical integrity than the proximal vas deferens. It is also reported that proximal vas deferens, their sperms and biochemical constituents, manifest differential sensitivity to different androgen and their metabolites in the adult and the pubertal animals (Prasad and Rajalakshmi, 1976; Chinoy 1984, 1985). The vas deferens passes from the scrota through the inguinal canal into the abdominal cavity over the urinary bladder to the urethra. Urethra is a tube connecting the urinary bladder to the exterior. In male, it passes though the penis and is flanked by three columns of excretile tissue.

The paired seminal vesicles empty into the vas deferens just before they join the urethra. Near its source in the urinary bladder, are the paired prostate glands (which in human are fused to form a single prostate). The prostate secretions pass into the urethra via two sets of short and thin ducts. Mucus alkaline secretions are provided by seminal vesicles and Cowper’s gland and a thin milky fluid with a characteristic odour is added by the prostate. Seminal fluid may contain glucose and fructose which are metabolised by the sperm. Acid base buffers and mucus materials that lubricate the passage through which sperm travel.

**STRUCTURE OF SPERMATOZOA**


Mammalian spermatozoa are small and motile and show a general uniformity in
their internal and external structure. The sperm which performs the function of carrying genetic material from the male to the oocytes, consists of two principal parts, such as the head and the tail. The tail consists of four components such as the neck, mid-piece, principal piece and end piece (Phillips, 1975). The cytoplasmic droplets is present in association with the mid piece of immature spermatozoa. Spermatozoa with cytoplasmic droplets indicate disturbances of sperm maturation in the epididymis (Menchini-Fabris, 1986). Silver nitrate can differentiate many of the gross morphological features of spermatozoa, including the acrosome, sub acrosomal region, perforatorium, post acrosomal sheath, neck, dense outer fibres of the core of the mid-piece, annulus, principal piece and end piece (Elder and Hsu, 1981). Silver nitrate staining pattern of spermatozoa have revealed both, species specific and strain specific differences, especially of the sperm head.

**The Sperm Head**

The head of a normal spermatozoon varies greatly in shape. It is ovoid in the bull, boar, bear and rabbit and hook like in the mouse and rat. The human sperm head appears as a flattered body. The main part of the head is occupied by the nucleus which largely consists of closely packed chromatin material. Chromatin is defined as the diffused, interface of chromosomes or as a poorly defined mass of genetic material. The genetic information or the complete set of hereditary factors contained in the haploid set of chromosomes is designated as ‘Genome’. The haploid nucleus is surrounded by a double walled membranous cap known as acrosome. The narrow region which connects the
sperm head with the middle piece known as the neck. It is the most vulnerable and fragile part of the spermatozoan. The middle piece contains the primary chemical energy exchange mechanism, mitochondria, in the form of a sheath around the mid piece. These organelles are arranged in a tightly coiled spiral, surrounding the contractile fibrils which provide locomotion and originate in the neck of the spermatozoa and pass through the tail. The acrosome as well as other component parts of the sperm are encased in an outer lining of plasma membrane. Essentially, all animal spermatozoa display a similar arrangement of parts, subtle structural differences exist but the overall morphology of spermatozoa from diverse animals is surprisingly uniform.

Nucleus

Miescher was the first to investigate the nucleus of the spermatozoon from salmon fish. The nucleus is composed of DNA conjugated with protein. The chromatin within the nucleus is very compact and no distinct chromosomes are visible. Several nuclei have incomplete condensation, with apparent vacuoles. The genetic information carried by the spermatozoon is 'coded' and stored in the DNA molecules, which is made up of basically of many nucleotides. The hereditary characteristic of the sperm nucleus includes the determination of the sex of the embryo as a result of the reduction division that occurs during spermatogenesis. The sperm contains only half the amount of DNA present in the somatic cell. Thus the spermatozoon is a haploid cell which can be differentiated into male (Y) and female (X) bearing gametes. Spermatozoa containing X and Y chromosomes, do not seem to vary in shape and dimension but are separated by different
techniques (Hafez 1980; Lamming, 1990).

**Acrosomal region**

Acrosomal region is covered by acrosomal membrane and is covered by plasma membrane. Acrosome is formed by the golgi apparatus during spermatogenesis. It is essential for fertilization. The acrosomal region is particularly rich in hydrolytic enzymes. Some of the important acrosomal enzymes are acid phosphatase, β glucuronidase, hyduronidase, acid proteinase, neutral proteinase (Acrosin), ATPase etc. Out of these acrosin is the most extensively studied purified and characterized enzyme. It is involved in the transfer of spermatozoa through cervical mucus. Another important function is its ability to bind to the zona pellucida (Topfer Petersen, 1987; Tesarik, 1988). Acrosin is apparently also involved in capacitation and acrosome reaction (De Jonge, 1989; Nuzzo, 1990). Acrosin determination is a useful parameter to predict the fertilizing potential of spermatozoa (Schill, 1991). Acid proteinase occurs in human spermatozoa in much lower amount than that in spermatozoa of other mammals (Sarkar, 1996; Mishell et al., 1997).

**Sperm tail**

The sperm tail arises from the spermatid parts. The centriole during spermatogenesis is differentiated into 3 parts: mid piece, main piece, and end piece. The mid piece is of a similar length as the head, is separated from the tail piece by a ring, the annulus. The mid piece possesses a cytoplasmic portion and a liquid rich mitochondrial sheath that consists of several spiral mitochondria, which surrounds the axial filament in
a helical fashion. An axial core consists of two central fibrils surrounded by a concentric link of nine double fibrils runs through the tail, a pattern common to cilia and flagella. It is made up of tubulin, dynein and other hexonemal proteins, involved in the flagellar movements i.e. sperm motility (Mann and Lutwak-Mann, 1981).

The sperm tail is well adapted for function related to motility. The nine larger outer fibrils of the tail, the main contractile elements are capable of propagating localized contractions along their length. The smaller inner fibers may be specialised for the rapid conduction of impulses, arising rhythmically at the neck and coordinating the localized contraction in the outer fibers (White, 1974).

The mid piece, a thickened region of the tail between the head and principal piece provides the sperm with energy necessary for motility. The central axial core of 11 fibrils surrounded by an additional outer ring of nine of

Individual mitochondria is wrapped spirally around these outer fibrils to form the mitochondrial sheath, which contains the enzymes concerned in the oxidative metabolism of the sperm. The mitochondrial sheath of the mid piece is relatively short, only a little longer than the combined length of the head and neck which connects the former (Gibbons, 1977; Hafez, 1980).

Sperm plasmalemma

Like other cells spermatozoa are completely surrounded by cellular membrane the plasmalemma. Several components adhere to the surface of the spermatozoon. Most of these surface components originate from seminal plasma but a few are innate to the
spermatozoon. Of the components derived from the seminal plasm are lactoferin, proteinase inhibitors, and decapacitation factor. The plasmalemma of a spermatozoon shows regional differences in biophysical and biochemical properties.

Sperm membranes possess protein, carbohydrate and lipids, making the essential composition of these structures a mixture for lipoglycoproteins. Lipids apparently function strictly as membrane components and stabilizers. Plasmalemma of spermatozoa plays a functional role in several phenomena such as sperm motility, capacitation and fertilization.

FEMALE CONTRACEPTIVE METHODS

Natural Family Planning

Although surveys indicate that the rhythm or calander method is the most extensively used method of natural family planning worldwide there has been little or no scientific evaluation of any suggested calander based rules for determining the period abstinence (WHO, 1998).

Natural family planning (NFP) requires two actions: Identification of the woman’s fertile period and abstinence from intercourse during that time. There are several methods of NFP, including calendar or rhythm method, the cervical mucus (or Billings) method, the basal body temperature method, and the symptothermal method. Each uses a different technique to predict periods of fertility and recommends different lengths of abstinence. The effectiveness of all NFP methods is dependent upon the couple’s motivation to prevent pregnancy and ability to interpret the symptoms of ovulation.
Traditional methods

In some regions women still rely on traditional methods of contraception; the United Nations estimates that approximately 77 million women use traditional methods to control their fertility. These methods may be actual services (for instance, vaginal sponges or wax based cervical barriers), substances (for instance, lemon juice douches), or behaviour patterns. Little research has been done on either the efficacy or the safety of these methods.

Female sterilization

Voluntary female sterilization is accomplished by surgically occluding the fallopian tubes so that the egg and sperm cannot meet. The methods used for female sterilization vary according to the surgical approach used to reach the tubes, the timing of the procedure, and the procedures used to occlude the tubes. Two approaches are commonly used to gain access to the fallopian tubes: (1) mini laparotomy which involves pulling the fallopian tubes through a small abdominal cavity and block the tubes. Both approaches are highly effective, with failure rates of less than one per 100 after one year (WHO, 1994).

Barrier methods

Barrier methods which include condoms, spermicides (foam, suppositories, tablets, cream, soluble films and jellies, diaphragms, cervical caps and sponges, act by mechanically or chemically preventing sperm from entering the uterus. Although use
effectiveness rates are lower for barrier methods than for hormonal or surgical methods. Barrier methods offer several advantages to both users and providers. The major advantage to users in the absence of long term side effects.

**Condoms**

The female condom or vaginal pouch is a new vaginal barrier method, currently available in the USA and several European countries. It is being promoted as a method that women can use to protect themselves from STDs and HIV infection. Its general acceptability and effectiveness, in both pregnancy and disease prevention, remain to be determined.

**Spermicides**

Spermicidal contraceptives consist of (1) a sperm killing agent and (2) base that distributes the agent over the cervix and physically blocks the semen from contact with the cervix. Commonly used spermicidal agents include nonoxinol, octoxinol, menfegol and benzalkonium chloride. The spermicidal agents are delivered in the form of foams, creams, jellies, melting suppositories, foaming tablets, soluble film, lubricated condoms and sponges.

**Vaginal barrier methods**

Vaginal barrier methods, such as the diaphragm, cervical cap and contraceptive sponge, prevent pregnancy by blocking sperm from entering the uterus and by holding
spermicide over the cervix. The effectiveness of vaginal barrier methods is dependent upon a variety of factors, including ability of the user to master proper use, user motivation near the time of intercourse. All female barrier methods can be inserted several hours before intercourse and must be left in the vagina for at least six hours after intercourse.

Short term side effects of these methods can include irritation caused by latex, the sponge material, or spermicides used with the method. Most vaginal barrier methods are not widely available in developing countries.

**Intrauterine device**

The intrauterine device (IUD) is a highly effective, safe reversible means of preventing pregnancy for selected women, particularly those who are not exposed to STDs and have already borne children. IUDs are small plastic or metal devices that are inserted into the uterus through the cervical canal. Although the precise mechanism of action is not known, it is hypothesized that the IUD interferes with sperm motility and ovum transport. Recent research suggests that the IUD's main mode of action is in the prevention of fertilization, thus contradicting the widely held belief that IUDs act as abortifacients.

IUDs are of two types medicated and non medicated. Medicated IUDs currently in use include two hormone releasing models, models that contain copper (Copper T 380A, Copper T 200, Copper T 220 C, Multiload 375, Multiload 250 and Nova T. Non medicate IUDs now in use are Lippes loop and single and double stainless steel ring. The
first hormonal IUD was progestasert. The net pregnancy rate was lower in women compared to those who used an inert device (Newton et al., 1979). The insertion of progestasert decreased blood loss in contrast to the use of Cu-IUDs (Rybo and Bergquist, 1976). Further studies resulted in development of IUD releasing more potent progestin levonorgestrel. The main mechanism of action is the suppression of endometrium which is uniform in the uterine cavity (Barbosal et al., 1990). The suppression of the endometrium is reversible after removal of the IUD and normal endometrium is established (Ortiz and Croxatto, 1987).

Accumulated data now indicate that while IUD users are more likely than users of some other contraceptives to experience an ectopic pregnancy, they are 50% less likely to experience ectopic pregnancy than women not using contraceptives. Copper containing IUDs carry the lowest risk of ectopic pregnancy while progesterone releasing IUD carry the highest risk. A commonly experienced side effect associated with both medicated and non medicated IUDs is increased volume of menstrual bleeding per cycle.

Hormonal Methods

Combined oral contraceptives use synthetic estrogen and progesterone to prevent pregnancy. Taken daily the hormones act to inhibit ovulation, alter the endometrial lining and impair sperm passage into the uterus by thickening of the cervical mucus. When administered in a specific post coital regimen, oral contraceptives can also act to prevent implantation of a fertilized egg. The primary known adverse effect of oral contraceptive use is increased risk of cardiovascular system diseases including thromboembolism,
stroke, hypertension, heart attack and atherosclerosis. Data from developed countries have shown that use of oral contraceptives protects against ovarian and endometrial cancer but may be associated with increased risk of breast and cervical cancers. Probably the biggest concern to many users of oral contraceptives is the side effects they experience especially in the first few months of use. These include irregular menstrual bleeding, nausea, mood changes, headaches, skin changes (chloasma), decreased sex drive, decreased vaginal lubrication and an increased incidence of cervical ectropion.

A number of progestogen only methods are in use or being tested worldwide. Progestogens used in these methods include levonorgestrel, medroxprogesterone acetate and norethisterone. Progestogen only methods, protect against pregnancy by thickening cervical mucus, changing the endometrium and often inhibiting ovulation. Progestogen only method may eliminate some of the risk associated with estrogen. While the major side effect of progestogen only methods is irregular bleeding, the total amount of blood loss generally remains the same. Users of progestogen only methods may experience prolonged bleeding, more frequent bleeding.

Minipills are less widely used form of oral contraceptives. The progestogen - only "minipill" does not contain estrogen and is slightly less effective than combined oral contraceptives. Minipill users have a higher risk of ectopic pregnancy than do users of combined organ contraceptives.

Norplant, a subdermal implant system that provides up to five years of contraceptive protection, consists of six silicone rubber capsules that are inserted under the skin of the woman's arm. Because widespread use of Norplant is relatively recent,
information about the long term safety of the method is limited to data collected from clinical trials. There is currently no evidence of increased cardiovascular disease among users. Some women using Norplant experience side effects. The most frequently reported side effect is a change in menstrual bleeding patterns including prolonged menstrual bleeding, bleeding or spotting between periods, amenorrhea or a combination of these side effects. Side effects that may be reported include headache, nausea, dizziness, acne, weight gain, breast tenderness, growth of facial hair and functional ovarian cysts.

Two long acting injectable contraceptives - depot medroxyprogesterone acetate (DMPA) and norethisterone enanthate (NE) have been approved. In addition to being convenient and non coitus dependent, the methods are highly effective. The result of recent study suggest that bone density may be reduced in long term users of DMPA. There is good evidence that use of DMPA provides protection against endometrial cancer. Use of DMPA appears not to be associated with either increased or decreased risk of ovarian cancer.

Once a month injectable contraceptives contain both estrogen and progestogen and are highly effective with failure rates of less than 1%. Some women prefer once a month injectables to long acting injectables because they produce regular monthly bleeding and cause less spotting. One major disadvantage of monthly injectable is the estrogen related side effects that some women experience (Puri and Van Look, 1994).
Post-Coital Methods

Post-coital methods are intended for emergency use only and are not recommended for use as a regular family planning method. They are highly appropriate in case of unplanned, unprotected intercourse, suspected contraceptive failure caused. For example, by a broken condom, dislodged diaphragm or missed pill, and rape or incest.

The most frequently used post-coital method involved administration of steroid hormones (estrogens or estrogen-progestogen combination) within 72 hours of unprotected intercourse. Hormonal treatment prevents implantation, probably by causing changes in the endometrium. A commonly used dose regimen (often called the Yuzpe regimen) consists of taking 0.1 mg of ethinylestradiol and 0.5 mg of levonorgestrel as soon after exposure as possible and again 12 hours later. Regimens containing ethynylestradiol and norgestrel, danazol, levonorgestrel or norethisterone have also been used. The side effects described for use of oral contraceptives also apply to use of post-coital hormone.

High dose estrogen

The first group of drugs used for postcoital contraception was estrogens in high doses. The main problem with this regimen is the high incidence of side effects. These include nausea, vomiting, breast tenderness and menorrhagia. Major side effects are uncommon but ectopic pregnancy occurs in about 10% probably because estrogen prevent intrauterine pregnancy better than ectopic pregnancy (Morris et al., 1973; Sparrow, 1974).

Cook et al. (1986) reported that injection of conjugated estrogens was also effective in preventing pregnancy after coitus. However, the necessity of using an i.v.
injection limits its use.

**Oestrogen-Progestogen Combination (Yuzpe regimen)**

Yuzpe et al. (1974) first reported the use of oestrogen progestogen combination for post coital contraception. To be effective, the treatment has to be started within 72 hr of intercourse. The advantage of this method is low dose of estrogen. However, the incidence of nausea is still over 50% (Yuzpe et al., 1982; Percival Smith and Abercombie, 1987). The action of Yuzpe is probably multifactorial. Histological examination shows the endometrial stroma undergoes more rapid maturation than the glands (Yuzpe et al., 1974). The effect on ovarian function is heterogeneous (Rowlands et al., 1986).

**Danazol**

Danazol is a semisynthetic steroid derived from 17α-ethinyl testosterone. It has been used for many years for treatment of endometriosis. Rowlands et al. (1983) first reported the use of danazol for post coital contraception (Rowlands et al., 1983). The incidence of nausea and vomiting were much lower than there of the Yuzpe regimen. This was shown by Zuliani et al. 1990. The main site of action of danazol was probably also at endometrium (Rowlands et al., 1986).

**Levonorgestrel**

Levonorgestrel has been evaluated for post coital contraception, both as a regular method and for emergency contraception. Kesseru et al. (1973) tested the efficacy of the
regular use in post coital contraception. The main problem was the high incidence of menstrual disturbances. Other side effects were infrequent. The WHO task force on post ovulatory methods for fertility regulation have recently completed a randomized comparison of Yuzpe regimen with levnorgestrel for emergency postcoital contraception (Ho and Kwan, 1993). It appears that the use of levonergestrel would be a better choice than the Yuzpe regimen in emergency contraception.

**Mifepristone**

Mifepristone is a progesterone antagonist at the receptor level. Because of its action on endometrium it has been used for post coital contraception. The initial results were promising. Glasier et al. (1992) compared the efficacy of the Yuzpe regimen with mifepristone for post coital contraception. The results supported by WHO task force on post ovulatory methods for fertility regulation (Webb et al., 1992) showed that mifepristone was more effective than the Yuzpe regimen with lower incidence of side effects. If these results are confirmed they may become the drug of choice for post coital contraception (Puri and Van-Look, 1994, WHO, 1998).

**ANTIFERTILITY VACCINES**

The immunological approaches are being developed to regulate fertility and are primarily directed towards the use of anti-hCG, and vaccination with zona pellucide glycoproteins. The initial vaccine was developed by linking the entire β-hCG to tetarius toxoid. But this vaccine had a large variability in the magnitude of the response.
Improvements were made by using a heterospecies dimer (HSD) instead of B-hCG. This vaccine is safe and free of side effects in women. Further developments are in progress (Buckshee, 1994).

Immunocentraception based on the human zona pellucide 3 (ZP3) glycoprotein is also being explored. The feasibility of this approach with respect to safety is being investigated by undertaking in vivo studies using marmoset monkeys. Efforts are also ongoing to generate the appropriate antigen such as recombinant ZP3. Whether this recombinant protein is competent to induce long-term infertility without adverse side effects is the subject of current investigations (Puri and Van-Look, 1994). Researchers are working on development of an improved version which will provide same and longer duration of protection with a single injection. Studies are also on for producing the immunocentraceptive that can be taken orally in the female (WHO, 1998).

PLANT PRODUCTS

Plant products constitute approximately 25% of all the prescribed medicine even in the most advanced countries like USA. The development of safe, orally reversible effective fertility regulating agents from plants for human beings is not a new idea. For centuries virtually every ancient culture has utilized plants as one form or other in an attempt to control population. The future prospects lie in plants and their products as potential source of drugs.

The history of fertility control can be traced back for 4000 years with discovery of a prescription for contraception written on an Egyptian papyrus which suggested the
local use of a paste containing ground Acacia (Havemann et al., 1967). Medicinal plants used for fertility regulation have been mentioned in Ayurvedic literature. Moteria Medica and Folklore. The Indian folklore medicine includes a large number of plants reputed as oral contraceptives and abortifacients. In ancient Japan 'misagami' a thin transparent paper disc made of bamboo tissue was placed in front of the cervix to prevent conception. In India the Kadamba fruit, the seed of red lotus, the plasa flower, the Samoli flower and the palm lead were used as oral contraceptive agents. Argemone (flower) Neem (seed) oil showed anti-implantation abortifacient actively in female rats (Nutan et al., 1988) Indian Medicinal plants associated with antifertility property are numerous (WHO, 1994; Chinoy et al., 1997; Rao and Alice, 1999).

The WHO task force antifertility plants tested in a multicentered effort 1000 extracts from 295 plants collected all around the world. None of these were followed up (Chaudhury, 1995). Similar work was obtained at the National Institute of Health, Bethesda and other centers carrying out work in this area. Some plants have been clinically evaluated for their antifertility activity. These include Vicoa indica, Montanoa tomentosa and a combination of Embelia ribes and Piper longum in females (Chaudhury, 1995; WHO, 1998).

The ethanatic extract of B. marginatum has been reported to possess abortifacient effect and also prevents implantation (Prakash et al., 1993; Janathan et al., 1995). Saraca indica is useful as a stimulent to the indometrium and ovarian tissue (Srivastava et al., 1984). Aloes compared from Aloe vera was found to be beneficial in cases of functional sterility and disturbed menstrual function. The saponin glycasid from Asparagus
*rasemoses* exhibited antioxytocic activity. *Syreplocos racemose* has been reported to be useful in treating uterine disorders (Kiritikar and Basu, 1987). *Boerhaavia diffusa* is known for its potent antiinflammatory property. The combination of *Saraca indica*, *Symplocos racemosa*, *Adhatoda vasika*, *Aloe vera*, *Asparagus racemosus*, *Boerhaavia diffusa*, *Bombax metabaricum*, *Cocos nucifera* and *Tinospora carditobia* known as U-3107 was shown to have uterine tonic activity in rats (Mitra et al., 1999).

**OVARIAN FUNCTIONS**

**Oogenesis**

The formation of a haploid ovum by meiosis in the ovary is referred to as oogenesis. Initial oogonia in the foetal ovary increases in number by mitosis. The process of oogenesis begins early in the foetal life of a mammal. It passes through a series of cellular transformations before it matures as an ovum ready for fertilization. At birth the ovary contains a definite number of primary oocytes which do not increase after birth. Actually the oocytes are in leptotene stage of the first meiotic division often described as a dictyate stage. At this stage they remain quiescent until puberty when hormonal stimulation induces them to resume meiosis.

At birth a mouse ovary contains about 8000 dormant oocytes arrested in the dictyate stage. At the first maturation division the chromosome number is reduced to haploid state. The cell division proper is unequal. Large part of cytoplasm and the other haploid set of chromosomes remain to form the secondary oocyte. The polar body may survive to undergo a second division with the second maturation division of the oocyte.
Figure 3: Oogenesis in mammals
or may degenerate. The second maturation division occurs either before ovulation or after the entry of the sperm just before fertilization. As a result of the second maturational division, another polar body is formed. If the first polar body has survived it may also undergo division coinciding with 2nd maturational division another polar body is formed. A spermatocyte produces four spermatozoa as a result of two divisions, whereas a primary oocyte produces only one functional female gamete the ovum (Fig.3).

Oogenesis and follicular development takes place ubiquitously. Follicle formation is initiated as a cluster of cells forming a separate entity. This constitutes the primordial follicle. The cell in the centre of this cluster of cells, destined to become the future ovum enlarges to become the oogonium and the other cells of the cluster arrange themselves as a layer around the oogonium. This is a primary Graafian follicle. The oogonium has a large spherical nucleus and a large amount of cytoplasm with deuteroplasmic constituents. As the oocyte grows the follicular epithelium increases by mitosis and another layer of cells membrana granulosa appears between the oocyte and the original follicular epithelium. The outer follicular epithelium derived from stroma constitutes the theca. The theca differentiates into an outer theca externa and an inner theca interna. A basement membrane called lamina propria appears between the theca interna and membrane granulosa. At this time the growth and differentiation of the follicle a zona pellucida is formed. Between the zona pellucida and the membrana granulosa is a space transversed by radiating protoplasmic projections called carona radiata. The cells of membrana granulosa from a projection lodging the ovum within it. It is called the cumulus oophorus. A large number of irregular lacunae appear among the granulosa cells and coalesce to
become the antrum. The surrounding membrana granulosa secretes a fluid into the antrum. The fluid content of the antrum is called the liquor folliculi. It contains proteins and hormones like FSH, LH, PRL and steroids, PGs and enzymes like ACPase, AlPase, ATPase, LDH etc. and proteoglycans.

Membrana granulosa becomes a site for estrogen secretion in the preovulatory follicles. The main endocrine component of the follicle is the theca interna which secretes estrogens. At the same stage in the development of the follicle the granulosa cooperates with theca interna in synthesizing hormones. Steroidogenic enzymes are present in the follicular layer and these follicles do produce estrogens. The mammalian follicle is very unique called a Graafian follicle. Three stages can be identified in the development of Graafian follicle, the primary Graafian follicle, consisting of a single layer of cells, a secondary Graafian follicle with more than one layer of cells and a tertiary Graafian follicle with two thecal layers, granulosa and the antrum. The theca interna is the endocrine compartment secreting estrogens and they have all the attributes of a steroid secreting cell. Hydroxysteroid dehydrogenases are localized in the theca interna.

The sequence of events in oogenesis in mammals can be summarized as follows:

1. Precluster stage.
2. Formation of primary graafian follicle with the primary oocyte in the centre.
3. Enlargement of the nucleus of the primary oocyte and squamous follicular cells becoming cuboidal.
4. Oocyte enlarges in size and follicular layer increase.
5. Appearance of the antrum as small spaces.
6. Formation of a single large antrum and initiation of the 1st maturational division in the oocyte.

7. Formation of a fully mature tertiary Graafian follicle.

8. Beginning of the 2nd maturation division and arrest of its metaphase plate.


**Ovulation**

With the maturation of the ovum on protrusion is formed by the follicle at the outer space of the ovary. This is called the preovulatory swelling. The expansion of the follicle causes the swelling. The different follicular layers at the region of the swelling become thin. The ovum and the cumulus oophorus surrounding it get detached and float in the liquor folliculi. A thin conical point appears at the centre of the swelling. This is called the stigma. The stigma bursts and the ovum with the cumulus oophorus and liquor folliculi are thrown out into the peritoneal cavity. The ovum is picked by the fimbrinae of the infundibulum and pushed into the infundibulum where the ovum awaits the arrival of the sperm.

**Corpus luteum**

The ovulated follicle does not regress and degenerate. The cells of the follicular layer undergo hypertrophy, large concentration of cytoplasmic droplets appears and organelles associated with steroid (progesterone) production develop. The new structure transforms itself into an endocrine organ called the corpus luteum. The hypertrophied cells
of this structure are called lutein cells and the process of its formation is known as luteinization (Sarkar, 1996).

**Uterus**

The uterus in rats is "Y" shaped with the lower limb being short. Anteriorly there are two horns. They are united at their caudal ends called corpus uteri. The two halves of the caudal ends and are separated by a septum composed of longitudinal muscle and connective tissue. The caudal end of the uterus is devoid of endometrium and is lined by cuboidal cells.

The two horns of the uterus are tubular in structure. The uterus is covered externally by a serosa and the inner wall is divisible into an outer myometrium and an inner endometrium. The myometrium forms the bulk of the histological structure of the organ. The uterine myometrium consists of an outer layer of longitudinal muscles and an inner layer of circular layer of muscles all of smooth muscle type with areolar tissue, blood vessels and lymph vessel and nerves. The mucosa is lined by a simple columnar epithelium separated from the endometrium by a lamina propria. The lamina propria contains small polyhedral cells with large round nucleus and clusters of lymphocytes. The endometrium contains the lamina propria, the uterine epithelium, the uterine glands and a number of blood capillaries. The polyhedral cells change into large decidual cells of the placenta during placentation. The uterine fluid is usually alkaline. There is no endometrium at the corpus uteri. It is lined by cuboidal cells. The middorsal and mid ventral wall of the corpus uteri is fused with the vaginal wall to form a trap like space.
either side known as fornix. The corpus uteri projects into the short vagina through the cervix. Cervix is lined by a stratified squamous epithelium. The human uterus is 7 cm in length and 5 cm in width at the upper end and 2.5 cm wide at the lower end. The uterine cavity is narrow dorsoventrally.

Histologically the frequency and patterns of myometrial contraction vary with different stages of the estrous cycle and menstrual cycle (Csapo and Sauvage, 1968; Behrman et al., 1969). Before estrous, the rat myometrium consists mainly of muscle cells and a few fibrocytes with the onset of estrus, fibroblasts predominate and myocytes contain an increased number of ribosomes and enlarged Golgi complex and sarcoplasmic reticulum. The role of myometrial contractions in intrauterine sperm transport remains unclear. In human, endometrium is composed of epithelium and stroma. A single layer of tall columnar cells lines the uterine lumen. The epithelium forms simple or branched tubular endometrial glands extending into the stroma, and occasionally through the myometrium. The endometrium varies in thickness from 1 to 6 mm, depending upon the stage of the menstrual cycle, and can be divided into three histologic zones. The zona compacta is the uppermost layer and is composed of compact, hypertrophied stromal cells. The middle layer, the zona spongiosa is made of large glands with little intervening stroma. The zona basalis is the deepest layer and contains the bases of the glands, surrounded by dense stroma.

The stroma of the endometrium contains various spiral arterioles which are terminal vessels surrounding the glands. Progesterone causes the stromal cells to become large, pale, and filled with lipid and glycogen.
Fluctuating levels of estrogen and progesterone throughout the menstrual cycle cause remarkable change in the structure and ultrastructure of the uterus and in the biochemical and physical characteristics of endometrial secretions (Hafez, 1980; Lobo et al., 1997).

**Vagina**

It is a fibromuscular tube, 6-8 cm in length in human. It is dorsoventrally flattened and opens posteriorly through an opening called vulva. At the anterior end of the vulva there is a muscular cylindrical clitoris. It is homologous to the penis of the male but does not contain any erectile tissue. Three distinct regions are distinguishable in the structure of the vagina. The external or posterior constructed opening, a dilated middle region and a narrow upper or uterine end. Vagina serves as a receptacle for sperms and a passage way for the conceptus. It represents the lower most or posterior part of the female reproductive system. It extends from the vestibule of the uterus.

In human it is located in front of the rectum and behind the urinary bladder. Vulva is a longitudinal slit like opening into which both the uterus and the vagina open. It is present at the tip of an eminence formed by a pad of flat lying over the pubis bone in the human female.

In the lower mammals mainly estrous cycle is found. It is mainly completed in 4-5 days. The vaginal epithelium undergoes changes controlled by the changing hormonal milieu and these changes are correlated to changes in uterine wall. The estrous cycle occurs in four stages in which characteristic changes occur in the vaginal smear. The
estrous cycle involves proestrous phase, estrous phase, metaestrous phase and diestrous phase.

The appearance of nucleated epithelial cells in the vaginal smear indicate the onset of the reproductive cycle or proestrous phase. The estrous phase is the period of heat or sexual receptivity and is characterised by presence of flat, irregular cornified scales, which are without nucleus. The metaestrous phase is characterised by the presence of scales, nucleated epithelial cells and leucocytes in the smear. In the diestrous phase, only leucocytes are seen.
RATIONALE OF PRESENT WORK

PART - 1

HORMONAL COMBINATION

Due to the uncontrolled population growth research on fertility regulating methods in human has been given high priority. There has been advances in contraceptive technology over the past few decades. However, the currently available contraceptives though still usable is inadequate to meet the present day requirement and the rapidly expanding future needs.

Research to develop state, effective reversible and acceptable methods of fertility regulation for men has been supported by several international agencies and many national research councils.

Hormonal methods are the most advanced of contraceptive methods for men. Their development was much to the involvement of Asian centers and investigators. The suppression of sperm production by hormonal means is achieved by the suppression of the secretin of both LH and FSH or of FSH alone and the maintenance of androgen levels in the physiological range. Various drugs either alone or in combination have been tried (Waites, 1993; Cummings, 1994). Two multicentered studies in which men suppressed to azoospermia or severe oligozoospermia were conducted by WHO to establish the contraceptive efficacy rates (WHO, 1990; WHO, 1998).

All hormonal regimens capable of suppressing sperm production to the same degree as in WHO studies (WHO, 1990; WHO, 1996) should receive high levels of
sustained and reversible contraceptive efficacy. Encouraging results of these studies have stimulated interest in the long acting drugs. For example, novel formulations of testosterone esters may be administered alone or in combination with progestagens such as DMPA.

**MEDROXYPROGESTERONE ACETATE**

Depot medroxy progesterone acetate (DMPA, Depo Povera; 17-alpha-hydroxy-6 alpha methyl progesterone acetate) is a progestagen and a derivative of progesterone. It is one of the most widely studied hormonal contraceptive drugs and best known type of injectable contraceptive drug, as its efficacy and safety have been repeatedly demonstrated.

MPA was first synthesized in 1958 (Babcock et al., 1958). It was microcrystalline suspension permitting prolonged action. It is a 6-Methyl progestin also known as potent progestational steroid which possesses antiandrogenic, syndrogenic and glucocorticoid activities when tested *in vivo* (Brown et al., 1979; Lin et al., 1978; Bullock et al., 1978).

**CHEMISTRY**

**PHARMACODYNAMICS**

Medroxyprogesterone acetate is a 17-acetoxy, 6 Methylpreg-4-ene-3, 20 dione. It belongs to class of C-21 steroids. It has a very close structural similarity to natural progesterone. Like progesterone, medroxyprogesterone acetate is thermogenic. It is a
Figure 4: Medroxy Progesterone Acetate (MPA)
progestational agent devoid of estrogenic activity. It is prepared as a sterile aqueous microcrystalline suspension for intramuscular injections. Each ml contains Medroxyprogesterone acetate 150 mg - polyethylene glycol 3350-polysorbate 80-sodium chloride - Methylparaben-propylparaben water for injection. The unusual stereochemistry of crystal structure seems to be important for its slow release (Duax et al., 1978) into the blood. Because it is not metabolized as rapidly as its parent compound, progesterone, MPA can be given in smaller amounts than progesterone with an equivalent progestational activity. DMPA, the long-acting injectable formulation of MPA, consists of a crystalline suspension of this progestational hormone. DMPA in appropriate doses suppresses pituitary gonadotropins and also suppresses the Leydig cell function in male i.e. suppresses endogenous testosterone production.

PHARMACOKINETICS

Following intramuscular administration, medroxyprogesterone acetate (MPA) is slowly released resulting in low, but persistent levels. MPA has a plasma half life of about 4-5 hours (Besch et al., 1966). The levels of MPA decline more slowly and could be detected even 200 days or more after a single injection (Ortiz et al., 1977; Fotherby et al., 1980) MPA is approximately 90 to 95% protein bound. MPA crosses the blood brain barrier and is primarily excreted in the faces via biliary secretion.

MODE OF ACTION

When DMPA was administered in appropriate doses it suppresses the secretion of
pituitary gonadotropin and also suppresses the Leydig cell function, i.e. suppresses the endogenous testosterone production. When DMPA was administered in large doses to male rats, ram or normal men it resulted in azoospermia which at the same time was accompanied by maintenance of accessory gland functions and libido (Frick et al., 1977; Sanchez et al., 1979). The decrease in production rate of testosterone after DMPA was reported by Rivarola et al. (1968) and Nolten et al. (1976).

In normal men, serum concentration of LH, FSH and testosterone had registered a decrease following treatment with DMPA (Rivarola et al., 1968; Faundes et al., 1981). Thus, the efficacy of DMPA might be due to its ability to suppress the circulating testosterone, for which three mechanisms had been proposed (Barbieri and Ryan, 1980): (1) by increasing the metabolic clearance rate of testosterone; (2) by decreasing the circulating gonadotropins and (3) by directly interfering with Leydig cell steroidogenesis. It was demonstrated that DMPA induced hepatic testosterone, a ring reductase activity in rat liver (Altman et al., 1972). Hence it was postulated that DMPA increased the metabolic clearance of testosterone. A similar increase was reported in normal men and woman (Gorden et al., 1970) but other experiments in men treated with DMPA failed to show an increase (Nolten et al., 1976). The decrease was due to the inhibition of gonadotropin secretin and by the direct inhibition of Leydig cell steroidogenesis. A decrease in the serum gonadotropin concentration by about 25-50% was reported in case of boys and men treated with DMPA (Rifkind et al., 1969; Meyer et al., 1977).

The direct effect of DMPA on Leydig cell steroidogenesis could be attributed to the fact that a large deduction in plasm testosterone without plasma LH or FSH being
altered, was noticed in a boy with hypothalamic hamartoma secreting LHRH, after the administration of DMPA (Judge et al., 1977) and the direct effect of DMPA on Leydig cell steroidogenesis could also be due to suppression of 17β-hydrosteroid dehydrogenase activity (Satyaswaroop and Gurpide, 1978; Rao et al., 1995; Roy and Rao, 1996). Similarly in vitro studies of Barberi and Ryan (1980) have also demonstrated.

**TOXICOLOGY**

The first toxicological studies on MPA were carried out on several hundred mice and rats. They were 100-200 times more than the human dose of MPA and were compared with animals receiving no drug. The mortality rate and the incidence of neoplasms were similar in both the groups and no death could be attributed to the drug (WHO, 1982). However, a decrease in the production rate of testosterone after DMPA was registered by Rivarola et al. (1968); Rao and Roy (1993) and Rao and Shah (1998). In addition to decrease in testosterone production, MPA may also increase the hepatic clearance of testosterone.

The toxicological review panel saw no reason to alter its opinion that MPA was safe for use in human beings (WHO, 1982). A large number of clinical trials including multicaentered studies organized by WHO has been carried out in many countries using MPA, thus making it probably the most intensively studied and the most widely used hormonal contraceptive preparation (Fraser and Holck, 1983).
METABOLIC EFFECTS

As recently summarised by Cullins, good epidemiologic evidence exists that use of DMPA reduces the risk of developing iron deficiency anemia, pelvic inflammatory disease. This contraceptive may also have beneficial effect upon hemotologic parameters in individuals with sickle cell disease. Like progesterone, medroxyprogesterone acetate is thermogenic. At very high dosage levels used in the treatment of certain cancers, corticoid like activity may be manifested. MPA had little or no metabolic efforts in relation to blood coagulation and fibrinolytic factors, platelet functioning, carbohydrate and lipid metabolisms in liver, renal and thyroid function etc. (Whigham et al., 1979; Astedt et al., 1971).

Because DMPA does into increase liver globulin production it is not associated with an alteration in blood cloting factors or angiotensinogen levels. DMPA has not been associated with increased incidence of hypertension or thromboembolism (Wilson et al., 1984). A WHO study reported that mean blood pressure measurements were unchanged in DMPA users two years of injection (WHO, 1983).

The oral glucose tolerance tests were performed on long term DMPA users and matched controls not using hormonal contraceptives (Lieu et al., 1985; Virutamasen et al., 1986). The mean glucose levels were slightly higher among DMPA users. Mean insulin levels were also higher. A slight deterioration in glucose tolerance among DMPA users is probably not clinically significant and returns to normal after use of DMPA is discontinued.

The findings of 11 studies that evaluated plasma lipid among DMPA users were
reviewed (Weshoff, 1996). Although little or no change was observed in mean triglyceride and total cholesterol levels, in all seven studies in which mean HDL cholesterol levels were measured. These levels were lower among the DMPA users. Of five studies in which LDL cholesterol was measured, three noted an increase among DMPA users. Therefore although the lipid changes with DMPA use are not beneficial no evidence to date suggests that they are associated with an acceleration of atherosclerosis.

Many investigators found little or no effect of MPA on liver function (Amatayakul et al., 1980). But the evidence from various studies is conflicting. In one study, MPA produced no significant change in aspartate aminotransferase, alkaline phosphatase, lactic dehydrogenase bilirubin or bromosulphthalein retention (Amatayakul et al., 1980) while other showed an increase in aminotransferase (Bajaj and Madan, 1983) and third group found no change in aminotransferase but an impairment of hepatic changes of bromosulphthelein (Avari, 1990) as a result of competition by the circulating progestogen for hepatic excretory mechanisms.

To determine the effect of DMPA on the hypothalamic-pituitary axis, Mishell et al. (1972) measured serum LH and follicle stimulating hormone (FSH) levels for two months after a single injection was given. Goldzieher et al. (1970) measured FSH and LH levels in single blood samples from women who received injections of DMPA every three months for as long as two years. In this cross sectional study, both FSH and LH levels remained in the mean range of those in the control. Therefore long term use of DMPA also does not cause complete suppression of the hypothalamic-pituitary axis.

Overall studies revealed that this MPA could be used in combination with
androgen supplementation (Rao et al., 1998; WHO, 1998).

**DIHYDROTESTOSTERONE (DHT)**

Testosterone may be regarded as prohormone. Testosterone is derived from the basic structure of androstene. All the androgens possess cyclopentano phenanthrene nucleus. Testosterone is the 17β hydroxy 3-one derivative of androgens. Testosterone in males is produced by Leydig cells, from acetate indirectly and form cholesterol directly. Testosterone is characterized by an axo group in position 3, and a hydroxyl group in position 17.

An increase in the duration of effectiveness of injected testosterone can be achieved by esterifying the molecule at 17 hydroxy position. Several esters have been synthesized of which testosterone propionate, enanthate, cypionate and buciclate have been used.

Testosterone is converted to 17β oestradiol. It is incompletely known what the biological relevance of these conversions are. The receptors for testosterone and DHT are identical but the binding of DHT is considerably stronger and therewith this conversion is a biological mechanism to amplify testosterone action. By esterification of this molecule at 17 position, its effects could be prolonged (Nieschlag and Behre, 1990). The structure of dihydrotestosterone (DHT) is given in Figure 1. Direct testicular secretion of DHT accounts for only 20-25% of the production rates found in the general circulation. The remaining 75-80% arises from peripheral conversion of secreted testosterone. The non-testicular production of DHT occurs in liver, kidney, muscle, prostate and skin, either
Figure 5: Structure of Dihydrotestosterone (DHT)
via. 5α reductase Type I (liver and non genital skin) or Type II (genital and male accessory gland tissues).

EFFECTS OF DHT IN MALE REPRODUCTION

Prenatally conversion of testosterone to DHT is a requirement for the formation of the prostate and the male external genitalia as evidenced by the syndrome of 5α reductase deficiency characterized by genital malformation. Beyond this stage of development this conversion is still a necessity. In subjects with 5α reductase deficiency development and function of prostate are subnormal. But approximately normal growth of the phallus, regulation and pigmentation of the scrotum and deepening of the voice are observed. It cannot be excluded that these actions are more efficacious when testosterone is converted to DHT.

Lotz and Krause (1981) reported that dihydrotestosterone is more effective than testosterone as a reversible antifertility agent in male rats. It is able to reduce circulating gonadotropins and androgen levels more effectively than testosterone in rats implanted silastic elastomers containing DHT. Testicular atrophy was also noted ensuring infertility in these animals after 6 weeks. However, the animals showed normal ejaculatory function as judged by the presence of vaginal plug in cagemates. Moreover accessory gland function was maintained while affected spermatogenesis was noted. DHT also induced a complete knock of gonatotropins thereby ensuring a state of hypophysectomy to affect spermatogenic activity (Ahmad et al., 1975; Harris et al., 1977). Ramakrishnan et al. (1989), reported the effect of dihydrotestosterone on pitutary, testicular and spermiogram
as well as accessory gland functions in male rhesus monkeys. The treatment brought about a decrease in LH, FSH and androgen levels. The ejaculated spermatozoa showed morphological abnormalities and decreased motility but, sperm counts were not significantly affected in treated male rhesus monkey, who had received 1000 µg DHT. Seminal fructose was reduced. However sexual behaviour was maintained. Histological functions of testis exhibited a decline in seminiferous tubular diameter, containing distorted cell population in them. The intertubular tissue became thick.

Rajalakshmi et al. (1990) also reported ultrastructural changes induced by dihydrotestosterone in monkey sperm. At high dose level (100 µg or 1000 µg). DHT induced displacement of mid piece, loosening of plasma membrane over head region and increase in electron density of acrosomal region. In view of aromatization of androgen to estrogens, causing gynecomastia and behavioural changes, studies on non aromatizable steroids like DHT and androstanediol and their esters have been suggested for induction of functional sterility in males (Puri and Van Look, 1994). DHT in combination with MPA has been suggested from studies carried out from our laboratory earlier (Rao and Roy, 1992; Rao and Shah, 1998).

PHARMACOKINETICS AND OTHER EFFECTS

The study on absorption of DHT through intramuscular injections to hypogonadal men revealed that DHT injections seem to be an effective and convenient technique for resolving serum physiologic DHT levels. This approach is suitable for long term substitution therapy (Diaz-Sanchez et al., 1989) in addition to other androgen replacement
therapies (Cantril et al., 1984).

Effect of dihydrotestosterene on hypothalamo pituitary testicular axis was studied by percutaneous administration of DHT to normal men. The result of this study revealed that DHT administration for 10 days to men has an inhibitory effect on the hypothalamo-pituitary-testicular axis (Kuhn et al., 1984). The rise in plasma DHT concentration was accompanied by a concomitant increase in 3α-androstanediol glucuronide and a fall in plasma testosterone and estradiol levels (Koremann et al., 1987).

SIDE EFFECTS

The lack of local or systemic side effects in all cases confirmed that percutaneous DHT administration is a relatively safe modality of androgen therapy. Moore et al. (1988) did not observe any side effects with DHT treatment. Twin studies have shown that elevated testosterone and DHT levels do not predispose to prostate enlargement or symptoms of benign prostate hyperplasia (Meikle et al., 1997). Moreover a negative effect of DHT in prostate pathophysiology is not proven. In view of the ability of aromatization of androgenic compounds to oestrogens and the possibility of such metabolized oestrogens to evoke unwanted side effects like gynecomastia and behavioural changes, this non aromatizable androgen (DHT) and its esters are chosen with progestin, medroxyprogesterone acetate (MPA) to find out the contraceptive efficacy of this regimen in male rats (WHO, 1998).
PART - II

PLANTS FOR FEMALE FERTILITY REGULATION

Apart from human costs in terms of mortality, morbidity and suffering, unwanted pregnancy can place a heavy burden on the health resources of poor countries. Although contraceptive use continues to rise in the world, the currently available methods, however good, do not meet all the different requirements of all the potential users. For example for reasons of personal, cultural or religious convictions some people are not able to use certain modern methods. Also, couples have different contraceptive needs at different stages of their reproductive lives and existing methods do not meet all the changing needs.

The first contraceptive technology revolution was needs-driven, with emphasis on methods that could have a demographic impact. When the revolution stalled, the poorly funded field became opportunity driven. In the second contraceptive technology revolution the field must again become needs driven with emphasis this time on the needs and perspectives of women. This does not mean ignoring demographic realities. Rather it means acknowledging that what women want for themselves in what the world needs for survival.

PHYLLANTHUS AMARUS (Fig.6a)

The genus Phyllanthus belongs to the family ‘Euphorbiaceae’. Phyllanthus is a genus of herbs or under herbs chiefly distributed in the tropical and subtropical region of the world. About 24 species occur widely in India and some ornamental exoties are
planted in gardens. The species for the study is *Phyllanthus amarus* which is known in different languages as:

- Hindi: Jangli amli, Jaramala
- Sanskrit: Tamalaki, Bhumyamalaki
- Gujarati: Bhonyaanmali
- Marathi: Bhuialavi
- Oriya: Bhuialo
- Urdu: Bhuiamla
- Kannada: Kirunelli
- Tamil: Kilanelli, Kilakkainelli
- Telugu: Nelausirika
- Malayalam: Kirganelli, Kizhanelli

**Distribution:** Throughout India as weed in cultivated and waste lands. The plant is found throughout the hotter parts of India from Punjab to Assam and southwards to Travancore, ascending the hills to 3000 ft (Hooker, 1979).

**PLANT**

**Stem:** Branched, annual glabrous herb. 30-60 cm high with slender, spreading leaf learing branchlets.

**Leaves:** Numerous, distichous, subsessile, often imbricating 6-13 by 3-6 mm, elliptic oblong, base rounded, petioles very short, stipules lanceolate-
subulate very acute.

Flowers: Yellowish, numerous, axillary, males in groups of 1-3, female solitary.
Sepals of male flowers 0.6 mm long, rounded, those of female 1-2 mm long, oblong, subacute with white margins, stamens 3, anthers sessile on a short column, styles minute, free two lobed

Fruits: 2.5 mm diameter depressed globose, smooth capsules underneath the branches

Seeds: Tri-gonous, pale brown, with longitudinal parallel ribs on back.

Parts used: Whole plant

Properties and uses:
The plant is bitter, astringent, cooling, diuretic, stomachic and antiseptic. The plant is used as a diuretic in dropsical affections, gonorrhoea and troubles of genito-urinary tract. An infusion of the young shoots is given in dysentery. The fresh root is said to be an excellent remedy for jaundice. The powdered leaves and roots are pulverized and made into poultice with rice water, used to reduce oedematous swelling and ulcers (Chopra et al., 1956). The plant is considered to be a de-obstruct, diuretic, astringent and cooling. A decoction of the plant is administered in jaundice or half an ounce rubbed up in a cup for milk is given morning and evening or the root or dried small bitter leaves in powder are used in teaspoonful doses (Nadkarni et al., 1953). The plant is stomachic, good for sores and in dysentery. The fruit is bitter, useful for tubercular ulcers, wounds, sores, bruises, scabies and ringworm infection. Parts of the plant are used to cure constipation. The plant
is very much used in blennorrhagia, dropsy and diarrhoea. The decoction of the root and leaves is bitter and is a favourite remedy for cure of intermittent fever (Kirthikar and Basu, 1933). The fresh leaves are also considered a remedy for jaundice (Sharma et al., 1979).

Extensive work has been done on *Phyllanthus amarus*, Linn, Ottow was the first to isolate a bitter principle which he named Phyllanthin and assigned C_{39}H_{37}O_{8} for its molecular formula. Krishnamurthy and Seshadri (1946) made a significant contribution in the study of the plant by Phyllanthin and hypophyllanthin extractions from its leaves, which are later identified as lignans (Row et al., 1964). Phyllanthin was found to be (+) 3, 4, 3', 4', 9, 9'-hexamethoxy-8, 8' butyrolingnan with absolute (8s, 8's) configuration (Row et al., 1966; 1967). Another new compound viz. linteralin was isolated (Ward et al., 1979). Two new securinega type alkaloids isobubbialine and epibubialine were isolated from leaves of *phyllanthus amarus* as well as the three known alkaloids phyllanthine, securinine and norsecurinine (Houghton et al., 1996). The structures of these unknown compounds were determined by means of UV, IR, mass and NMR spectroscopy. The petrol extract of *P. fraternus* showed antifungal activity against *Helminthosporium sativum* (Bhatnagar et al., 1961). The leaf extract showed antifungal activity against *Alternaria alternata* (Bhowmic and Chowdhary, 1982). The plant has been reported to possess antifungal, antibacterial and antiviral activities. *Phyllanthus amarus* (Syn. P. niruri) is widely used in Ayurvedic medicine for the treatment of liver ailments and has shown both in vitro and in vivo activity against hepatitis B. virus (Houghton et al., 1996). In clinical trials for hepatitis in 160 children it showed no side effects (Dixit and Achar, 1983).
Phyllanthin and hypophyllanthin, lignan constituents isolated from *Phyllanthus amarus* when tested on various cancer cell lines did not show significant cytotoxic effect on any cell line.

Biochemical effects of *Phyllanthus amarus* was studied after oral administration of the drug to rats with respect to diabetes (John and Krishnaswamy, 1993). Alcoholic extracts of roots and leaves of *Phyllanthus niruri* showed hepatoprotective effects in experimental rats (Agarwal et al., 1988). *Phyllanthus debilis* and *Phyllanthus amarus* were studied and no substantive hepatoprotective effects were found in these herbs (Subramaniam, 1995). It has been reported that *Phyllanthus amarus* is a potential diuretic, hypotensive and hypoglycaemic drug for humans (Srividya and Periwal, 1995). The extract of whole plant have exhibited spermicidal activity in vitro and also reduced fertility rate in mice (Rao et al., 1997a,b; Shah, 1995).

**BALANITES ROXBURGHII (Fig.6b)**

The genes Balanites belongs to the family "Simarubaceae". *Balanites aegyptiaca* or *B. roxburghii* is an evergreen tree occuring is a wild state in Arabia, Egypt, East and West Africa. Burma and India (Asholkar and Chadha, 1979), which is known in different languages as follows:

<table>
<thead>
<tr>
<th>Language</th>
<th>Translation</th>
</tr>
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<tbody>
<tr>
<td>Gujarati</td>
<td>Begarea</td>
</tr>
<tr>
<td>Hindi</td>
<td>Hingan, Hingot</td>
</tr>
<tr>
<td>Marathi</td>
<td>Hinganabet</td>
</tr>
</tbody>
</table>

78
It is a spiny tree about 6 meters in height with glabrous branches ending in a very strong sharp ascending spines (Kapadia, 1975). The intermode between the thorn is about 1.5 cm. long. Thorns are strong green with brownish up to 4 cm. long and often show 1-2 nodes on which bifoliate leaves and inflorescences are found. The leaflets are dark green in colour, lanceolate, oblong or obovate with obtuse or sub-acute apex. Surface is smooth to hairy (in small young leaves) and with entire margin (Rastogi and Mehrotra, 1993).

The flowers are small white or greenish white cymes 4-10 flowered. Flowers are sweet smelling and bitter. Cure 'vata' and 'kapha'.

Fruits are 4.2 to 5.4 cms long and 25 to 3.8 cms broad. They are dark green in colour when immature, changing to greyish yellow when ripe during the winter month. They are one seeded, 5 grooved drupes with hard and woody endocarp. The fruit wall of unripe but fully grown fruit of Egyptian origin which is smaller than Indian fruits has been found to contain 4.08% diosgenin/yamogenin (in 44:56 ratio) on dry weight basis (Hardman and Sofowara, 1972).

Unripe fruits are cathartic. Ripe fruits are used for whooping, cough and skin troubles. The bark, unripe fruit and leaves are pungent, bitter and purgative and are considered to have anthelmintic properties. The seeds yield a fatty oil (43%) used for burns and freckles and for soap making (Ambasta 1992).

Clonal propagation of *B. roxburghii* by stem cutting and air layering techniques
has been reported by Amalraj (1987; 1992). Ghanim (1991) also reported the methods of vegetative propagation, flowering, fruiting. Hingota oil, oil from seeds, variation in diosgenin, buil extraction, oil cake as animal feed and the industrial potential of *B. roxburghii*. A new saponin with insect antifeedant activity has been isolated from the stem bark of *B. roxburghii* reported by Jain (1987). Jain and Banerjee (1988) found oleate linoleate, palmitate and stearate from seed oil of *B. roxburghii*. The oil was found non-toxic but repellent (Possible due to sterol-rich nature) to the domestic vermin *Piriplanata americana*.

Saponins and sapogenins isolated from *B. roxburghii* were also studied for insect antifeedant activity against the larvae of the noctuid. *Spilosoma obliqua* (Jain and Trivedi, 1991). In this study the most active compound was diosgenin - 3-0 (alpha - L-rhammopyranosyl (1-2)-beta-D-glycopyranosyl (1-3) beta - D - glycopyranosyl (1-4)) - beta - D - glucopyranoside (x), inhibiting approximately 73.63% feeding at 0.02% concentration. They were also assessed relative efficacy of all the tested compounds, using azadirchtin as standard.

Saponins from *B. roxburghii* was also investigated asa mosquito larvicide. LC50 and LC90 values and the nature of active constituents were studied (Zarroug et al., 1990) in different stages of mosquito larvae. The larva of *Anopheles arabiensis* were more susceptible than *Culex quinnefaciatus* and *Acedes aegypti*.

Saponin extracted from fruit kernel was also tested against second and fourth instar larvae of three mosquito species and LD50 and LC90 values were determined. They concluded that this saponin was more active than the water extract indicating it an
active compound for larvicidal effects. Pharmacological studies with bark of *B. roxburghii* was done by Siddiani et al. (1991).

Intravenous administration of the ethanolic extract of fruit pump of *B. roxburghii* produced a triphasic response on the blood pressure of anaesthetized dogs and cats (Shrihari Rao et al., 1988). Prominent antidiabetic activity from an aqueous extract of mesocarp of fruits of *B. roxburghii* was reported (Kamel et al., 1991). In addition to known spirostanol glycoside, balanitin - 3 and new sapogenol, 6-methyl diosgenin, a new furostanol saponin, balanitoside has been isolated from fruit (mesocarp) of *B. roxburghii* (Hoshny et al., 1992). Pericarp alcoholic extract of *B. roxburghii* has been demonstrated to have spermicidal action on rat sperm (Rao et al., 1997a). The extract also possessed reversible antifertility affect in rodents (Rao, et al., 1997a,b).

From the information cited above, it is an urgent need to explore and expedite a possible family planning method of hormone pill and/or herbal product for developing countries like India where population growth is an unresolved problem. Hence, this study was undertaken to investigate the contraceptive effect of hormonal combination and plant products in rodent models.