CHAPTER I

INTRODUCTION
In considering the approaches to male contraception, several features of male reproductive physiology have special relevance. Today human population is growing faster than the rate at which the basic needs of each individual could be met. If the present trend of alarming population growth rate continues, it is expected to double itself in the next 45 years. Out of the total projected world population of 6.2 billions in 2000 AD, the population of the developing countries is expected to 4.5 billions. The World United Nations has estimated that world population will continue to grow for another 110 years and is projected to stabilize at 10.5 billions by the end of next century (Puri and Van Look, 1994). Over population has affected the society at all levels, in social, economic and political concepts of life, influencing thereby not only the physical standard but the very quality of living. In India if the population will keep growing at the present pace of 2.08%, it will become double in just 35 years.

Thus, the growth of global population has alarming accelerated in the present century. World population is increasing at a rate of 90 million or more a year, of which about 19% around, is in India itself. Therefore, even if the present impressive growth rate is maintained in 90's and subsequent decade, more than 15% of the urban population and 25% of the rural population will still be below the poverty line in 2021. The growing needs for medical services, food, fuel, shelter, environment, education and employment will further create enormous pressure. To overcome this problem, contraception has become necessary.
The need of family planning is not a recent phenomenon in human history. It can be traced to the very beginning of man's time on the Earth. During evolution itself, man has been equipped with a number of ingenious biological mechanisms for fertility regulation. The initial research and studies in this field were carried out using exclusively the female as an ideal model as the fertilization takes place inside the female. Although the balance is now being redressed. There has until recently been much less research on development of male contraceptives than on those intended for women. Furthermore, the need to maintain libido and potency in the male is paramount and the close morphological and functional relationship of spermatogenic and androgenic functions makes many methods unacceptable (Shain and Pauersten, 1980; Puri and Van Look, 1994).

The methods for fertility regulation such as periodic abstinence, withdrawal and condoms were oriented upto the second half of the twentieth century. With advent of steroid contraception, IUDs, diaphragms, spermicides, abortifacients, tubal ligation and induced abortion, the major burden of birth control has been transferred to women in the last 25 years. Thus, too long has the burden of contraception been placed on the shoulders of women and it is not fitting in today's new atmosphere of equal rights for women that the male has been called upon to take his share of the responsibility. Therefore, research has been channeled in other direction i.e. males. For the success of any family planning program, the male partner must assume more responsibility, and should share benefits and risks of whatever the contraceptive strategy the couple may opt for. This serves as a reminder of the need for male fertility control and the renewed interest in developing novel reversible methods for contraception for men in recent years has been stimulated by a number of factors, which allows
men to share more evenly the responsibility as well as the benefits of an effective family planning.

Any method for male fertility control should be

i) effective to a degree similar to existing female methods.

ii) acceptable to both the partners.

iii) effective.

iv) no any side effects in next pregnancy and

v) reversible (Nieschlag et al., 1981).

Today "the family planning" is the immediate and the most appropriate step against this "monstrous evil" of population growth. The explosive growth in the world population makes it imperative that a safe and reversible means of fertility control must soon be found to meet with the wide range of conditions encountered relating to health, community, attitude, family relationship, economic and social positions and other factors which influence the adoption of methods of fertility control.

Some progress has been made in the last few years which has considerably improved the future prospects of male contraception, although optimal male methods equivalent to those being employed in female are still to be devised (Zatuchni et al., 1986; Knuth and Nieschlag, 1987).

It is possible that, before the year 2000, two new methods of male fertility regulation may be developed: one based on suppression of sperm production with infrequent injections of male hormones, either alone or combined with
other gonadotrophin-suppressing agents and the other is a more easily reversed method of vas occlusion (WHO, 1993).

METHODS FOR MALE CONTRACEPTION
SOME OLD METHODS

The development of methods for male fertility regulation has been lagged well behind that of females. However, very few methods are practised in males.

TRADITIONAL METHODS

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

COITUS INTERRUPTUS AND PERIODICAL ABSTINENCE

The coitus interruptus and periodical abstinence are considered to be the common, easy methods of male contraception. However, the possible adverse psychologic effects followed by a high failure rate are often associated. The average failure rate is also very high, i.e. about 10-15/100 women-years.
CONDOMS

The condom is an effective, nonsystematic method of long range family planning that provides a safe means of prolonging intercourse and premature ejaculation. In recent years, acceptability of the condom has been increased due to the increased concern of AIDS and other sex diseases. The condoms are primarily a temporary contraceptive method and these have a limited shelf life, particularly in tropical countries.

VASECTOMY

Vasectomy has emerged as leading method of contraception. In 1950 and 1960 voluntary sterilization became popular through vasectomy. The earliest attempt to interfere with male reproductive system was to prevent disease rather than conception. Interruption of the vas deferens was thought by many to cure prostatic hypertrophy (Wolfers and Wolfers, 1974). The main advantage being that it is a simple technique of sterilization and is widely applicable to developing countries. Over 90 million have chosen sterilization as a means of fertility control, and the number of successfully performed operations show a steady increase and will continue to increase rapidly, until an acceptable physiological method to control fertility in the male is secured. Vasectomy may be accomplished by surgery or by the application of vas occlusive devices or some chemical occlusive substances. The studies raised questions about the possibilities of the post-operative effects (Hafez, 1980). The report ranges marked degeneration of germinal epithelium with an associated proliferation of testosterone secreting Leydig cell functions. Recently Flickinger et al. (1993,
Handelsman, 1994) reported severe inflammation of the epididymis after vasectomy due to the interstitial reactions.

Studies on rodent models, dogs, bull and human beings revealed that vasectomy does not adversely affect the weight of pituitary, gonadotrophin activity, physiology of testis, epididymis and spermatogenesis (Singhal et al., 1977; Chinoy et al., 1978; Chinoy and Chinoy, 1984). Chinoy et al. (1983) reported that there is no change in semen biochemistry and its androgen levels even after 20 years of vasectomy. Vasectomy does not adversely affect the general health or endocrine status (Peng et al., 1987; Tang et al., 1988). However, the pregnancy rate, following reversal is considerably lower, a consequence possibly related to the development of antibodies to sperm (Ansbacher et al., 1972) which is due to rupture of epididymis tubule, following destension (Bedford, 1976). In some cases control studies suggested that vasectomy may predispose to prostate cancer, whereas other studies found no increase in risk (Guess, 1990). WHO (1993) suggested an association both vasectomy and manifestation of a possibly preexisting testicular tumor. Several studies revealed no side effects of vasectomy and it is effective and microsurgical techniques are available to restore potency in over 80% of cases. The added advantage is the availability of present of recanalization or vasovasostomy (WHO, 1994). Since vasectomy reversal is neither cheap, reliable nor widely available, vasectomy must be considered as a permanent form of sterilization until more reversible methods supplant those presently used (Handelman, 1994).
The development of vas occlusive procedures includes extra luminal device and intraluminal device were practised. Non-surgical techniques which avoid a skin-incision are likely to be more acceptable and may be easier to reverse (Waites, 1988). Intraluminal devices implanted either with or without cannula include cylindrical plugs, spherical heads or threads to satire material, whereas in extraluminal devices a significant portion of the devices protrudes out side the vas deferens. Two main factors limit the acceptability of vas occlusion: one is the necessity for a skin-incision, which is unacceptable in some cultures. Non-incision vasectomy involves special clamp and other procedure with the percutaneous injection of a sclerotising solution (n-butyl-2-cyanoacrylate and phenol) into vas deferens. It is Chinese method and described by Li and Zhu (1985) and the other is the lack of certain reversibility. Xiao (1987) reviewed chemical methods for vas occlusion in use in China and described the use of medical grade polyurethane elastomeres (MPU) to form plugs. This later method appears to offer the possibility of easy reversal. Zhao, (1990) has been established a method based on the percutaneous injection into the restricted portion of the vas deferens of liquid silicone to form plug. Vas occlusion was induced by a single injection of ethanol, prostaglandin and ascorbic acid into the vas deferens and was found to induce sterility in male rats (Chinoy and Chinoy, 1984). Lead salts and hormones have also been used as vas occlusive agents in males (Rao, 1991; 1992).

Chinese investigators have led to two major technical improvements: The isolation and ligation of vas deferens through puncture in the skin and percutaneous injection into vasal lumen, of sclerosing or occluding agents
through a hypodermic needle (WHO, 1994). These would be useful to perform this technique in an easy manner.

**EXPERIMENTAL APPROACHES TO MALE CONTRACEPTION**

The final objective in the field of research in male fertility regulation is to develop a safe, effective, reversible, non-expensive and acceptable method as cited earlier.

**IMMUNIZATION AGAINST FSH AND LH**

Findings of Lumenfeld and Eshkol (1969) revealed that antibodies can be prepared to human FSH, which have little or no cross reaction with LH or hCG. From these results, possibility of immunological contraception for men based on inactivation of FSH by combination with antibodies has been tried. In rats, Raj and Dym (1976) demonstrated that antiserum to LH causes spermatogenic failure presumably by disrupting testosterone production. For fertility regulation, administration of immunogen (active immunization) which elicits antibody mediated and/or cell mediated immune response (Talwar, 1986; Hearn, 1980; Diczfalusy, 1986) or administration of antibodies (passive immunization) brings about neutralization of the native hormone action.

The fertility regulating vaccines against hypothalamic, pituitary and gonadal hormones, as well as sperm, ovum, conceptuses and placental antigens are being explored. Although active immunization against FSH did have an early inhibitory effect, it eventually disappeared with time (Wickings et al., 1980; Srinath et al., 1983; Nieschlag, 1986). On the other hand, passive and active
immunization of bonnet monkeys resulted in azoospermia or oligospermia leading to infertility. However, with the exception of FSH, these approaches represent unattractive propositions because of the considerable risk of autoimmune damage and/or major endocrine disturbances (Moudgal and Rao, 1985). Studies supported by ICMR which involves active immunization against FSH in bonnet monkeys resulted azoospermia. Some times, large number of spermatozoa were produced, but semen quality was poor (Moudgal et al., 1987). Thus, the influence of FSH on the functional maturation of sperm is an important consideration which might provide a lead for male contraceptive development (Moudgal et al., 1994).

INHIBIN

There is a water soluble substance of testicular origin, called inhibin. It has been postulated to regulate follicle-stimulating hormone (FSH) secretion through negative feed back. Thus, the inhibin with similar properties has been obtained from different sources, viz. rete testis (Setchell and Shrinathsinghji, 1972; Setchell and Jacks, 1975), semen (Franchimont et al., 1975) and extract of testis (Lee et al., 1974). It has been shown that inhibin may have several sites of action viz. direct effects on anterior pituitary (Steinberger and Steinberger, 1977; De Jong et al., 1979; Eddie et al., 1979). Possible systemic effects on the hypothalamus (Moodbidri et al., 1981), as well as local effect on the testis (Rich et al., 1979; Demoulin et al., 1981) are also reported.

As there is no convincing evidence of FSH suppression by inhibin, a line of research led to the discovery of other small peptides in the testis possibly active in regulating testicular functions (Nieschlag et al., 1981; Sharpe et al., 1981).
In India, Sheth and his colleagues have been carried out extensive work on inhibin. Inhibin interferes with the binding of FSH to testicular receptors which may result in reduced C-AMP formation by testicular tissue in response to FSH. The potential of inhibin like peptides of testicular origin for male contraception has been suggested (Sheth, 1994). Sheth and his colleagues collaborated with others to determine the amino acid sequence of a material. They designated as β-inhibin (Seidah et al., 1984; Johanson et al., 1984).

Inhibin caused sperm agglutination, impairment of cervical mucus penetration, sperm egg attachment, and also the spermatogenic inhibition in rats (Mehta and Sheth, 1992). The data also suggested that inhibin bioactivity does not have the expected inverse relationship with FSH (Simpson et al., 1987; McNeilly et al., 1988) and many have a more important role as an intragonadal regulator. Passive immunization of adult rats, hamster and marmoset with anti-seminal inhibin resulted in complete or partial block of fertility (Sheth et al., 1992).

In view of these findings, inhibin can offer a solution to the selective suppression of seminiferous tubular function without affecting Leydig cells which seems to be important. The increase of interest for inhibin as a contraceptive is important as the administration of such substance might produce infertility without impairment of libido. Recently Sheth (1994) reported that prostatic inhibin peptide (PIP) is a sperm coating antigen and is useful for immunocontraception in the male.
POST-TESTICULAR CONTRACEPTIVES

Many chemical compounds with reversible post-testicular effects on sperm stored in the epididymis have been described, but all have been discarded because of toxicity (Ray et al., 1991). Alpha chlorohydrin and the 6-chloro - 6-deoxy sugars were among the more interesting and better explored (Ford and Waites, 1986). These studies at least established that the principle was attainable and, at antifertility doses, demonstrated the ideal characteristic of a post-testicular drug. A variety of other compounds and their analogues are currently under investigation by various agencies, e.g. sulphasalazines, imidazoles, pyrimethamine (Puri and Van Look, 1994).

PLANTS AND PLANT PRODUCTS

As a matter of fact, plants are almost the exclusive source of drug for the majority of the world population today. Plant products constitute approximately 25% of all the prescribed medicine even in the most advanced countries like U.S.A. The development of safe, orally, reversible effective fertility regulating agents from higher 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 (Waltes, 1988).

The history of fertility control can be traced back for 4,000 years, with the discovery of a prescription for contraception written on an Egyptian papyrus on the method suggested was the local use of a paste containing ground Acacia (Havemann et al., 1967). The Indian folklore medicine includes a large number of plants reputed as oral contraceptives and abortifacients. In ancient Japan 'misugami' a thin transparent paper disc made up of bamboo tissue was placed in front of the cervix to prevent conception. In India, the kadamba fruit, the seed of the red lotus, the plasa flower, the samoli flower and the palm leaf were used as oral contraceptive agents. The plants that have reputation as a folklore or those which have been tested for antifertility activity constituents have been discussed. (Fransworth et al., 1975; Oswiecimska et al., 1980; Nagarajan et al., 1982; Fransworth and Bingel, 1985; WHO, 1994.

The antifertility effect of Ocimum sanctum 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 plumbagin on spermatogenesis and accessory reproductive organs of rat (Santhakumari et al., 1980) and Puevaria luberosa were also investigated (Daftari et al., 1981).
Nagarajan et al. (1982) have tested numerous indigenous plants having potential spermicidal activity in human beings and/or in animals. The green flower extract of *Malvaviscus conzattii* exhibited antifertility effect in male albino mice (Verma et al., 1980). Aqueous and alcoholic extract of *Embelia ribes* affected male reproductive organs and exhibited antifertility activity (Krishnaswamy and Purshothaman, 1980).

The crude seed extract of *Arbus precatorius* Linn., caused testicular lesion (Baijal et al., 1981). Rao (1987) also reported the antifertility effect of aqueous and alcoholic extract these seeds on fertility of males. The cattle grazing on an exclusive diet of *Leucana leucocephala* became infertile (Holmes et al., 1981). Chronic administration of *Sapindus trifoliatus* fruit extract caused testicular lesions and inhibited the process of spermatogenesis at primary spermatocyte stage in gerbils (Dixit and Gupta, 1982). Dixit et al. (1983) showed antispermatogenic activity of *Gloriosa superba* in male gerbils. The antifertility effect of the barries of *Solanum Xanthocarpum* (Solanaceae) evoked attention. Solasodine obtained from the barries affected the male genital tract of dog. Its chronic administration caused testicular lesion and spermatogenesis arrest (Dixit and Gupta, 1982). Rao (1986; 1988) obtained similar results using alcoholic extract of this plant seeds in rats.

An alcoholic extract of the outer seed coat and seed powder of *Bulea monosperma* showed significant antifertility activity at a dose of 200 mg/kg body wt. (Mehta et al., 1983). Sperm agglutination activity of extracts from roots of *Arum moculatum* and *Arum orientale* was studied by Maldenov (1982). The extract is believed to react specifically with receptors situated on the surface of the tails of human spermatozoa. The aqueous extract of *Echeneria*
gibbijlora has immobilizing and agglutinating effects on human spermatozoa (Huacuja et al., 1985). Reversible antifertility effects of Azadirachta indica leaves on mice reported (Mendulkar et al., 1981). 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 has also screened for their antifertility activity (Chinoy et al., 1994; Lohiya et al., 1992). Rao (1989) has reported a definite antifertility effect of the alcoholic extract of Terminalia bellirica fruit in male rats.

The work on the Tripterygium wilfordii would give some hope for the positive results (WHO, 1992) after the work on gossypol and its derivatives. It has been shown that a multiglycoside extract of this plant, caused reductions in sperm motility and concentration in male patients (WHO, 1994).

**HORMONAL METHODS FOR MALE FERTILITY REGULATION**

The endocrine approach to male fertility regulation is based on the suppression of pituitary gonadotrophin secretion. Research has been shown that the testis depends on the stimulation by pituitary hormone for maintenance of spermatogenesis and steroidogenesis. Both FSH and LH are required for spermatogenesis and that the action of LH is most likely manifested by the stimulation of Leydig cells to produce and maintain a high intratesticular concentration of testosterone. Numerous approaches are available for modifying pituitary secretion of these hormones and thereby disrupting spermatogenesis (de Kretser, 1976). FSH and LH have to be suppressed.
simultaneously, necessitating the need of testosterone substitution to maintain androgenicity (Nieschlag et al., 1989; Nieschlag and Habenicht, 1992).

**HORMONAL CONTROL OF SPERMATOGENESIS**

Spermatogenesis is a continuous process during which millions of genetic messengers are produced daily from the stock of primitive precursor cells in the seminiferous tubules in the testis. The complex cytodifferentiation of the germ cells is made up of mitotic divisions of the spermatogonia, meiotic division of the spermatocytes and the transformation of the non dividing spermatids into spermatozoa in the process known as spermiogenesis. Unlike the oocyte, spermatozoa carrying the genetical information of the male, have to be motile in order to travel the female genital tract and to be able to recognise the egg, penetrate its investing membranes and eventually fuse with it. Thus, spermatozoon is one of the most highly specialised cells in the body. Spermatogenesis is critically dependent on an adequate amount of testosterone being available to the Sertoli and peritubular cells of the seminiferous tubules. These cells intum, through ill-understood mechanisms, promote normal germ cell division and development (Sharpe, 1987). Although the exact amount of testosterone required to maintain quantitatively normal spermatogenesis is continuous (Rommerts, 1988), testosterone is an essential endocrine factor for gamatogenesis in males. The other hormone associated with spermatogenesis is follicle-stimulalting hormone (FSH). A schematic representation of the endocrine relationships between the brain and the testis is shown in Fig. 1.
FSH acts directly on the germinal epithelium (Means et al., 1976), while luteinizing hormone (LH) exerts its influence via testosterone produced by Leydig cells.

ORGANISATION OF SPERMATOGENESIS

The process of spermatogenesis which takes place in the seminiferous tubules of the testis - comprises a series of events leading to the development of diploid spermatogonia into haploid spermatids. The spermatogenesis is a lengthy chronological process whereby a small population of spermatogonial stem cells maintain their own numbers and cyclically furnish the cells that mature into spermatozoa (Fig. 2). The stem cells are formed the type A spermatogonia and they remain quiescent in the intact cells. These cells resume proliferation after damage occurs to the spermatogonial cells (Van Alphen et al., 1989).
The classification of the spermatogenic stages and their relative duration in the spermatogenic cycle (temporal succession of all spermatogenic stages) is derived from the pioneering work of Clermont (1972) who discovered that the periodic acid Schiff's reagent staining of the acrosome could be used to classify and number the spermatogenic stages. The spermatogenic cycle and stage duration is species specific and varies among the animals studied between 8 to 12.5 days (Clermont, 1972; de Rooij et al., 1986). The longest stage duration (16 days) was found in the human (Heller and Clermont, 1964). The evolution of testicular sperm from spermatogonia requires approximately 4-4.6 spermatogenic cycles (Clermont, 1972). From this observations the total duration of spermatogenesis was estimated to be 51-53 days in the rat, 37-42 days in the nonhuman primate and 74 days in man. In addition to the varying
duration of spermatogenesis in rats, non-human primates and men it is important to note that considerable differences exist with regard to the longitudinal arrangement of spermatogenic stages along the seminiferous tubule. In the rat stage I is followed by stage II, stage II by stage III and so forth. The spatial arrangement of stages has been described as spermatogenic wave (Perey et al., 1961).

When interpreting the effects of testosterone on germ cell development, it is important to distinguish between three phases of spermatogenesis: initiation, maintenance and reinstitution of the spermatogenic process. Initiation relates to the first completion of spermatogenesis, i.e. formation of testicular spermatozoa at the time of puberty. Maintenance encompasses the requirements during ongoing spermatogenesis in the sexually mature organism. Reinitiation refers to the restart of spermatogenesis once this process has been disrupted.

TESTOSTERONE AND INITIATION OF SPERMATOGENESIS

Although the neuroendocrine triggers during the neonatal juvenile, prepubertal and pubertal period still remain largely unknown, it is undoubtedly clear that during these phases the testicular functions are governed via the pituitary gonadotropins released in response to hypothalamic gonadotropin-releasing hormone (GnRH) (Plant, 1985).

Administration of androgen LH, or hCG in immature rats subjected to hypophysectomy or estrogen treatment, had a beneficial influence on germ cells but did not start complete spermatogenic development. Germ cell formation did not pass beyond meiotic stages or the appearance of early spermatid (Chemes
et al., 1976; 1979). LH only partially prevented germ cell loss induced by hypophysectomy, studied by Russel et al. (1987). Thus, in rat testosterone alone is not sufficient for initiation of the complete spermatogenesis process. However, testosterone alone can initiate the development of elongated spermatid in the primates (Chemes et al., 1982; Steinberger et al., 1973).

TESTOSTERONE AND MAINTENANCE OF SPERMATOGENESIS

The ability of testosterone to maintain the spermatogenic process represents the most intensively studied aspect of the hormonal regulation of spermatogenesis. The majority of investigations, particularly in rat, employed hypophysectomy as a means to eliminate the endogenous gonadotrophin supply. Other paradigms included, administration of estradiol or testosterone, and immunisation against LH. More recently, antagonistic analogues of GnRH came into use.

Administration of FSH to hypophysectomised or estrogen treated immature rats or selective immunisation against FSH clearly underscored the importance of FSH in the initiation of spermatogenesis process (Almiron et al., 1984; Kerr and Sharpe, 1985). The proposed target cells were spermatogonia, spermatocytes and spermatids, more importantly. Complete initiation of germ cell development was only achieved when testosterone (or LH) and FSH were combined (Yasuda and Johnson, 1985).

Administration of exogenous testosterone to hypophysectomised animals, either at the time of surgery or within a few days thereafter, produced a pronounced stimulatory effect on spermatogenic maintenance. In qualitative terms, all
aspects of germ cell development were supported by testosterone (Barlett et al., 1989; Rivier et al., 1980; Santulli et al., 1990). However, quantitative maintenance of the spermatogenic process, assessed from enumeration of germ cells in histological sections, was not achieved in any of the above mentioned studies. Sun et al. (1989) reported the estimation of daily sperm production served as the end point of evaluation. The effects of testosterone were dose dependent but diminished with prolongation of treatment. However, in contrast to these observations, testosterone alone maintained the daily sperm production over a 4-weeks period in estradiol suppressed animals (Robaire and Zirkin, 1981). Similarly DHT and 5-α androstanedione are also effective in qualitative maintenance of spermatogenesis (Dube et al., 1988). Concomitant administration of testosterone maintained spermatogenesis in a quantitative manner over a period of 30 days (Rea et al., 1986). It may be due to stimulation in pituitary and serum FSH. In contrast to rat model testosterone did not stimulate FSH in GnRH antagonist treated primates (Barlett et al., 1989). Thus testosterone alone maintains spermatogenesis quantitatively but not quantitatively in rat.

The information about testosterone and maintenance of spermatogenesis in humans is based on trials with androgens for male contraception (Nieschlag et al., 1989) and the studies of Matsumoto (1989) who used androgens combined with selective gonadotrophin replacement for the studies of hormonal regulation of spermatogenesis. In recent clinical trial with 19-nor testosterone, a less potent androgen than testosterone, azoospermia could be induced at a rate of 80% without impairment of other androgen dependent functions (Behre et al., 1989). Thus, the androgen type might be important for contraceptive method.
In the hypophysectomised rat model, it was soon recognised that the ability of testosterone or LH to reinitiate or restore spermatogenesis was rather limited (Huang et al., 1987). Dihydrotestosterone was less effective than testosterone (Harris et al., 1977). Chowdhary and Steinberger (1975) however, reported complete restoration of sperm count following testosterone administration. Hypogonadotropism and spermatogenic involution were induced by estradiol treatment for 8 weeks (Awoniyi et al., 1989a) or immunised against LH or GnRH for 10 week in animals (Awoniyi et al., 1989b). Thus, testosterone is not as effective in reinitiation as in maintaining the spermatogenesis process in rats and primates (Weinbauer et al., 1986).

Administration of LH to hypophysectomised patients restimulated spermatogenesis to the level of spermatocytes only (Mancini, 1969). Similarly, in hypogonadotropic patients qualitative but not quantitative spermatogenesis was achieved with hCG (Matsumoto, 1989).

REGULATION OF EPIDIDYMAL FUNCTIONS AND SPERM MATURATION

Spermatozoa entering in the ductuli efferents from the rete testis are functionally immature. They can neither fertilize ova nor able to initiate progressive motility. In the last decades of the 19th century, it has been noted that spermatozoa from the distal epididymis had greater motility potential than those from proximal segment of the duct. In the following years, other investigators reported that spermatozoa undergo physiological and
morphological alterations as they pass through the epididymis (Turner and Howards, 1977). After classic studies, of Young (1931) it became clear that spermatozoa mature during their sojourn in the epididymis. It is now known that both the spermatozoa and their fluid microenvironment undergo numerous changes along the length of epididymis (Bedford, 1975; Turner, 1979). The extent to which sperm maturation depends on the milieu in the epididymis on the one hand and intrinsic processes programmed into the sperm cell DNA on the other is still debatable. The chemical mediators of this maturation process are still largely unknown.

Regulation of both the epithelial and lumenal functions is under complex hormonal control. Though androgens, as in particular 5-α reduced metabolite of testosterone, i.e. 5-α dihydrotestosterone, are considered to be the primary modulators of epididymal functions, i.e. regulation by factors entering the lumen of the epithelium (Robaire and Hermo, 1988). Specific proteins secreted by the epididymis have been identified by several workers. Differences in staining pattern of Lectin binding in different epithelial cells also indicated the production distinct glycoproteins (Arya and Vanha-Perttula, 1984) which are androgen dependent (Vanha-Perttula and Arya, 1985). Lea et al. (1978) identified an acidic epididymal glycoprotein (AEG) having a molecular weight of 33,000. Forward motility protein (FMP) (Acott and Hoskins, 1978). Protein DE (Brooks and Higgins, 1980). Protein IX (Jones, et al., 1980), 32 K Protein (Wong et al., 1981) and the 37,000 dalton glycoprotein (Olson and Orgebin-Crist, 1982) have been identified. These proteins alter surface membrane properties of the spermatozoa (Jones et al., 1983; Burgos et al., 1985).
Jones and Glover (1973) indicated that, in the presence of androgen, the lining cells of the cauda epididymis maintain a constant milieu in the lumen of the tubule as a result of their capacity for absorption and secretion. The regulation by androgens of intermediary metabolism in the epididymis has been studied extensively by Brooks (1981).

The transport of ions across the epididymal epithelium is an energy-dependent process, has also been found by Wong and Yeung (1977) to be dependent on androgens. The system responsible for transporting inositol and carnitine across the membranes of epididymal epithelial cells have also been shown to depend on androgens (Yeung et al., 1980; Pholpramool et al., 1982). Finally, the synthesis and secretion of a number of epididymal glycoproteins and activity of number of enzymes are to a large extent mediated by androgens (Jones et al., 1980; Rastogi et al., 1979). Direct evidence that dihydrotestosterone is pivotal for spermatozoa to acquire their fertilizing potential has been obtained by assessing the effects of inhibitor of 5-α reductase activity in adult acutely castrated in male mice (Cohen et al., 1981). Recently, it has been demonstrated that the microenvironment surrounding epididymal 5-α reductase is crucial for its activity (Cooke and Robaire, 1985; Wong et al., 1981).

In experimental animals, testosterone is the primary androgen in the seminiferous tubule whereas dihydrotestosterone is the major androgen in the epididymal fluid. There is a decrease in the concentration of androgens and androgen binding protein (ABP) along the length of the epididymis (Howards, 1983). In general it is not known whether intraluminal DHT bound to ABP or testosterone from the blood is the critical source of androgen for the epididymal epithelium. However, it is thought that the initial segment of the epididymis is
dependent on intralumenal androgens and cannot be maintained by exogenous androgens (Fawcett and Hoffer, 1977). Androgens and other larger molecule cannot freely diffuse from the blood into the epididymal lumen because of a barrier to passive transport at specialized epithelium cell to cell tight junctions. Turner et al., (1981) show that radiolabeled testosterone and DHT in the blood are transported into the epididymal fluid but reach concentrations of only 30 to 40% of those in the blood.

The first report that exogenously administered testosterone was transformed to dihydrotestosterone and bound to a cytosolic protein in rat epididymides (Blaquier, 1971). The human epididymis in organ culture responds to both testosterone and dihydrotestosterone, with an increase in cell weight and protein synthesis (Tezon and Blaquier, 1981). In addition to the demonstrated actions of androgens on the excurrent duct systems, there are number of other hormones e.g., estradiol, prolactin and Vit. D. for which receptors have been identified. Very little information is known about the physiological functions mediated by such hormones in this tissue (Robaire and Hermo, 1988).

Transport of spermatozoa seems to be only indirectly dependent on the presence of androgens, since neuronal input to the epididymis is the apparent main driving force propelling the lumenal content down the excurrent duct system. Both acquisition of fertilizing ability and storage of spermatozoa have been found to directly depend on androgens (Orgebin-Crist et al., 1975).
STRUCTURE OF SPERMATOZOA

The biology of mammalian spermatozoa plays a pivotal role in the field of reproduction unlike other body cells. The sperm is unique in character. The life span of this fascinating cell is short and on purpose. It is endowed with the capability of existence as a foreign body in another individual. This capacity reflects the pronounced intra-cellular division of labour of the male gamete which is unmatched by another mammalian cell. It is dynamic in activity and continual change characterizes its life span. Nature has designed it for performing a specific role so as to achieve at fertilization, the transfer of genome and to serve as a nuclear bridge between generations. Having fulfilled its mission, it ceases to be a separate entity but leaves a permanent imprint on the future generation.

Early modern synthesis of the biology of mammalian spermatozoa were provided by numerous investigators (Lamming, 1990). Mammalian spermatozoa are small and motile and show a general uniformity in their internal and external structure. The spermatozoon, 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 in present in association with the mid-piece of immature spermatozoa. 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 fibers 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 and rabbit and hook-like in the mouse and rat. The human sperm head appears as a flattened body. The main part of the head is occupied by the nucleus which largely consists of closely packed chromatin material. Chromatin is currently defined as the diffused, interface of chromosomes or 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 entirely surrounded by a double-walled membranous cap, is 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 spermatozoon. The middle piece contains the primary chemical energy exchange mechanism, mitochondria, in the form of a sheath round the midpiece. 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 sperms 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. 65% of the head 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 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 gamates. 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-glucoronidase, hyaluronidase, 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. Acid proteinase occurs
in human spermatozoa in much lower amount than that in the spermatozoa of other mammals. ATPase is found on the outer acrosomal membrane.

**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 consisting 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 axonemal proteins, involved in the flageller movement 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 specialized 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 is surrounded by an additional outer ring of nine of coarse fibrils. Individual mitochondrion 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 midpiece is relatively short, only a little longer than the combined length of the head and neck, which connects the former (Gibbons, 1977; Hafez, 1980).

The principal piece (main piece), the longest part of the tail, provides most of the propellant machinery. The coarse nine fibrils of the outer ring diminish in thickness and finally disappear, leaving only the inner fibrils in the axial core for much of the length of the principal piece (White, 1974). The fibrils of the principal piece are surrounded by a fibrous tail sheath, which consists of branching and anastomosing semicircular strands or “ribs” held together by their attachment to two bands that run lengthwise along opposite sides of the tail.

**SPERM PLASMALEMMMA**

Like other cells, spermatozoa are completely surrounded by a cellular membrane, the plasmalemma. Several compounds 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 plasma are lactoferrin, 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 of lipoglycoproteins. Lipid apparently functions strictly as membrane components and stabilizers.
Plasmalemma of spermatozoa plays a functional role in several phenomena, such as sperm motility, capacitation and fertilization.

**LH-RH ANALOGUES**

Normal synthesis and secretion of pituitary gonadotrophins are dependent on appropriate stimulation by the hypothalamic decapeptide, leutinizing hormone releasing factor (LHRH). In recent years synthetic peptides with similar to that of gonadotrophin releasing hormone appear to be promising lead in male fertility control (Waites, 1994). Analogues of LH-RH can be used to abolish gonadotrophin secretion by over-riding this normal pattern of control. LH-RH has been shown to have two major effects either as potent LH-RH agonist or as LH-RH antagonists. A number of structural modifications of LH-RH have resulted in its superpotent agonist which have a prolonged action on the gonadotrophins of the pituitary (Belanger et al., 1985; Lunglmayr et al., 1985), and also has a direct effect on the testis (Seguin et al., 1980). Chronic administration of LH-RH and its analogues has been shown to cause testicular and prostatic atrophy (Sandow et al., 1978). Clinical studies to investigate the possible use of some of the LH-RH agonistic analogs for male fertility regulation have also been carried out (Waites 1994; Nieschlag et al., 1985). Toxic manifestations were also discovered with LH-RH analogues (Waites, 1980).

LH-RH agonist was found to suppress the pituitary secretion of LH, FSH and testosterone and to have a direct effect on the testis. A small scale study in the rhesus monkeys showed the complete reversibility after 20 months suppression
Doelle et al. (1983) demonstrated reversible inhibition of spermatogenesis in man by administration of LH-RH agonist (D-Trp^6, Pro^9 - Net). Oligospermia or azoospermia occurred in normal volunteers treated for 4 weeks. But, this method is quite impractical because of a pronounced reversible fall in plasma testosterone concentration together with a decline in libido and potency. LH-RH analogs are used for inhibition of spermatogenesis, while testosterone is administered exogenously to minimize the effect that would result from the lack of this hormone (WHO, 1992). On the other hand Gn-RH antagonists act directly on Gn-RH receptors and inhibit both pituitary and testicular functions. Hence, androgen supplementation is required to minimise these side effects (Rajalakshmi, 1994).

**STEROID HORMONES**

Sex steroids are known to suppress the spermatogenesis in animals and human beings by inhibiting pituitary gonadotrophin secretion. Heller et al. (1950) have established that increased gonadal steroid hormone level can, by negative feed back, inhibit FSH and LH secretion by the pituitary thereby disrupting spermatogenesis. Thus, from a number of studies, both FSH and LH can be suppressed by using androgen alone or in combination with progesterone or estrogen, which is an attempt to suppress spermatogenesis without interfering libido or potency.

**ESTROGENS**

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; Johnson and Gomes, 1977; Rao and Chinoy, 1984; Rao et al., 1993; 1994) showed its effects on male reproductive system. It has been reported that the estrogen treatment to mice manifested in epididymal maturational changes in spermatozoa as a result of androgen deprived effect (Rao and Mathur, 1987). Estrogenic compounds have been used as a potent antispermatogenic and antifertility agents in males (Rao and Chinoy, 1983). The potential contraceptive efficacy of a combination of testosterone and estradiol-17 β has been suggested by Ewing et al. (1977). The results suggested that the rats rendered azoospermic 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).

Many estrogenic compounds like diethylstilbesterol (DES) suppress spermatogenesis in man (Jackson and Jones, 1972), but the treatment caused abnormalities in reproductive tract (Thomas et al., 1985). Estradiol benzoate (E\textsubscript{2}B) showed androgen antagonistic and antifertility effects in rats (Chinoy and Rao 1982; Rao and Chinoy, 1983; 1984). It was concluded that E\textsubscript{2}B interfered with the microenvironment of the epididymis and had androgen deprivation effect on the target organs. The beneficial role of androgen on the toxic effects induced by DES were reported by Rao et al. (1993; 1994). However, etrogens 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

There are compounds that induce an appropriate histological changes in the uterus of an experimentally prepared animals. Progestin have been used as antifertility agents in the male, due to their antispermatogenic potential (Bennet, 1974). After being tested for their antispermatogenic properties they were classified in to following categories.

(i) PROGRESTERONE 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.

(ii) 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. Medroxyprogesterone 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 Δ6 progesterone) did not exert antispermatogenic effect in rats (Karkun and Kar, 1965).
The antispermatogenic properties of progestagens are exceedingly variable and dependent on the amount and the 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 results in loss of libido and potency as well as increased nipple pain. Hence, the application of simultaneous androgen therapy would be preferred (Roy, 1994).

**ANTIANDROGENS**

Most estrogens and progestogens are antiandrogens. Any gonadotrophic substance too can be considered as antiandrogen which reduces stimulation of Leydig cell testosterone synthesis. That means antiandrogens are compounds which prevent the expression of biological activity of androgens at target sites by inhibiting one or more of the following mechanism, viz.

a) intracellular conversion of testosterone into dihydrotestosterone (DHT)
b) receptor binding of DHT
c) translocation of the receptor - DHT complex to the nucleus
d) binding of hormone receptor complex to the acceptor site and
e) transport of newly synthesized messenger RNA (m-RNA) from the nucleus, by binding to a ribonucleo protein.
It means the second type of antiandrogens 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 feedback on hypothalamus (Brotherton and Harcus, 1973). But, Morse et al. (1973) suggested that cyproterone administration directly inhibits testosterone secretion and affects libido and potency. So if cyproterone alone was undesirable for male fertility regulation. Accordingly, these antiandrogens can not be used for fertility regulation (Neumann et al., 1978).

Many other substances have both peripheral androgen-antagonism and anti-gonadotrophic properties Cyproterone acetate (CPA) (Neumann et al., 1985) belonging to class of antiandrogens with additional progestational properties were considered to be suitable for male fertility regulation. Cyproterone acetate (CPA) was shown to produce reversible inhibition of spermatogenesis and reduction of plasma testosterone. CPA acts at the epididymis and affects sperm maturation. Rajalakshmi et al. (1976) and Moltz et al. (1980) achieved selective inhibition of sperm maturation in cauda epididymis by using micro doses of CPA without affecting the libido and spermatogenesis. However, clinical studies does not seem to support these results (Koch et al., 1976; Roy et al., 1976). In human, all studies reported, indicate a definite inhibitory effect on spermatogenesis (Wang and Yeung, 1980). Studies with a combination of cyproterone acetate and testosterone were also carried out (Kaur et al., 1992). However, this approach seems to be unfeasible as the antiandrogens are known to affect the adrenal function (Lee, 1974).
ANDROGENS

Although the inhibitory effects of androgens on spermatogenesis was demonstrated in laboratory animals almost forty year ago, their potential for male contraception has been extensively investigated mostly during the last few decades. Reddy and Rao (1972) used testosterone proprionate 50 mg daily to normal men and they demonstrated suppression of spermatogenesis first time. Earlier reports were also available on suppression of spermatogenesis by testosterone (Heller et al., 1958). Similarly, azoospermia condition was observed by testosterone administered through sustained released capsules in rabbit (Ewing et al., 1973) and rat (Reddy and Prasad, 1973).

Long acting esters of testosterone also induce 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). Currently the most studied preparation is the long-acting ester, testosterone enanthate (TE) and it is found to cause a marked suppression of spermatogenesis at a dose of 200 mg. Attempts are being made to design a spacing regimen where maintenance of azoospermia could be achieved with less frequent administration (Noble, 1977). The sperm production is diminished by 90% or more in dose dependent manner during administration and the volume of the ejaculate is usually unchanged (Cunningham et al., 1979; Mauss et al., 1978). After 34-48 weeks this inhibitory effect on spermatogenesis is completely reversible and reaches to normal levels (Paulsen, 1980). 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, is provided by studies using a 19-nor testosterone ester with long half life than testosterone enanthate (Knuth et al., 1985; Behre et al., 1990).

A single im. injection of testosterone trans 4-butyl-cyclohexylcarboxylate (20 AET-1) administered to castrate male cynomolgus monkeys (Weinbauer et al., 1986) could maintain the serum testosterone levels in the physiological range upto 4 months and appears to be 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 a use in male contraception (WHO, 1994).

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 used alone. A whole host of different gestagens were studied in combination with testosterone and its esters in a series of clinical trials sponsored by the Population Council. It was found that azoospermia could be induced in only a proportion of subjects with relatively high dose (Shearer et al., 1978).
Various hormonal regimens have been tested for suppression of spermatogenesis, sufficiently to act as an effective contraceptive (Knuth and Nieschlag, 1987; WHO, 1992; 1994).

From these various combinations the most effective was 200 mg depot medroxy progesterone acetate (DMPA) with 200 mg testosterone enanthate (TE) monthly. The incidence of side effects was low. However, there is some concern regarding the long-term metabolic effects of DMPA (Meyer et al., 1985; Friedl et al., 1985). But, combination tested so far, it seems that DMPA (200 mg), testosterone cypionate (250 mg) monthly, seems to be most promising combinations (WHO, 1979). It was shown that a long-acting ester of 19-nortestosterone (19 NT) alone (Schurmeyer et al., 1984; Knuth et al., 1985) or combination with DMPA (Knuth and Nieschlag, 1987) was more effective in producing azoospermia than testosterone enanthate. Rao and Roy (1993) and Rao et al., (1995a) demonstrated altered sperm function in rats administered with a combination of MPA + TE in rats. Same results were also documented by Wu and Aitken (1989) in hormonally suppressed oligospermic men with altered sperm fertilizing capacity.

RATIONALE OF THE PRESENT WORK

SECTION I

HORMONES FOR FERTILITY REGULATION

Due to uncontrolled world population growth, research on fertility regulation methods in human has given a high priority. The possibility of contraception in men by pharmacologic means has received little consideration in comparison with the enormous efforts devoted to this approach in women. One of the
reasons for this disparity is undoubtedly the absence in the male organism of a
simple phenomenon comparable to ovulation in the female which can be
manipulated with reasonable directness. Another, perhaps equally decisive,
aspect is that the technique of inhibition of gonadotropin release, so effective in
including functional infertility in women, is associated with the unacceptable
effect of potency reduction in men (Lotz and Krause, 1981). Moreover, any
acceptable antifertility pill for the male should be safe, capable of reversibility,
suppressing the sperm production or sperm function without interfering with
libido and potency or any other health status of men (WHO, 1992). Recently,
through increased public awareness statements supporting research on male
methods and the greater involvement of men in reproductive health have been
forthcoming from several quarters, including the women's health movement
(WHO, 1992; 1994).

The hormonal suppression of spermatogenesis is currently being investigated
as one of the reversible methods of male contraception (WHO, 1991). The
suppresssion of sperm production by hormonal means has been a general
research strategy for all agencies interested in male contraception. This
strategy has involved:

(i) the suppression of the secretion of gonadotrophins, either of both LH
and FSH or FSH alone;
(ii) the recovery of circulating androgens to physiological level without
restimulation of spermatogenesis and
(iii) the assessment of the functional capacity of residual sperm, if the
treatment fails to achieve azoospermia in all cases.
Exogenous steroids like androgoen and progestin are known to suppress the spermatogenesis by inhibiting the pituitary gonadotrophin secretion (WHO, 1994. Puri and Van Look, 1994). A variety of hormonal regimen includ-ing androgen with or without progestin were tried through out the world (Waites, 1988; Knuth et al., 1989).

A combination of androgen and progestin would be preferred, as the androgen administration compensates for the loss of endogenous androgen production and maintains the libido (Knuth and Nieschlag, 1987; WHO 1991).

**DEPOT MEDROXYPRGESTERONE ACETATE**

Depot medroxyprogesterone acetate (DMPA, Depo Provera) is a clinically important 6-methyl progestin is one of the most widely studied hormonal contraceptive drug 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 a 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). A review of numerous publications, which recorded studies of this progestational contraceptive formulation have given a leading role in contraception (Fraser and Holck, 1983).
CHEMISTRY

The formula of MPA is 17-acetoxy, 6-methyl-preg-4-ene-3, 20-dione. It belongs to the class of C-21. It has a very close structural similarity to natural progesterone. It is prepared as a microcrystalline suspension for intramuscular depot injections. The unusual stereochemistry of crystal structure seems to be important for its slow release (Duax et al., 1978) into the blood. It has also been suggested that the formulation procedure and the size of the microcrystals are critical for optimal prolongation of the duration of action (WHO, 1992). The structure of MPA is shown in Fig. 3.

PHARMACOLOGY

Microcrystal of MPA has a very slow solubility in body fluids and this provides prolonged release from the surface of the crystals at the depot site. MPA has a plasma half life of about 4 to 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). These characteristics contribute a very high contraceptive efficacy.

ARREST OF MALE FERTILITY

MODE OF ACTION:

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 function and libido (Frick et al., 1977;
FIG. 3. MEDROXYPROGESTERONE ACETATE (MPA)
Sanchez et al. (1979). The decrease in the 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; Faundez 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 (Barbierti 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 the 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 women (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 secretion and by the direct inhibition of Leydig cell steroidogenesis. A decrease in the serum gonadotrophin 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 plasma 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 the suppression of 17β-hydroxysteroid dehydrogenase activity (Satyaswaroop and Gurpide, 1978; Rao et al., 1995a). Similarly in vitro studies of Barberi and
Ryan (1980) showed an inhibition of testosterone production in rat interstitial cells. Chronic administration of DMPA to male rats increased 5α/5β reduction of testosterone in the liver (Albin et al., 1973). It has previously been shown that the presence of excess quantities of DMPA led to increased accumulation of dihydrotestosterone (DHT) and reduced formation of 5α-androstan-3α, 17β-diol when testosterone was incubated with rat prostate fragments (Patwardhan and Lanthier, 1975).

Sunde et al. (1982) demonstrated that DMPA was a potent inhibitor of 3α-hydroxy steroid oxidoreductase activity in the rat testis, epididymis, kidney and adrenal glands. Wentworth and Terner (1979) suggested that MPA specifically inhibited 3α-hydroxysteroid oxidoreductase without effect on 5α-reductase and 17β-hydroxysteroid oxidoreductase of germ cells.

The report on selective binding of gestagens on human sperms by Cheng et al. (1981), and their inhibitory effect on sperm motility in vitro (Hyne et al., 1978) as well as Cheng and Boettcher (1979) suggested a possible direct influence of MPA on sperm motility. However, Knuth et al. (1989) suggested that MPA acts on sperm motion characteristics via an altered function of the epididymis.

Gunsalus et al. (1980) and Bardin et al. (1981) suggested that MPA and probably other progestin altered the normal release of ABP from the Sertoli cells. Later Lobl et al. (1983) also suggested that during MPA treatment, testicular and epididymal ABP content declined in parallel with organ weights and hormones of FSH and LH whereas serum ABP concentration increased.
TOXICOLOGY

The first toxicological studies on MPA were carried out on several hundred mice and rats. They were given 100 to 200 times more than the human dose of MPA, and were compared with animals receiving no drug. The mortality rates and the incidence of neoplasms were similar in both 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) and Rao and Roy (1993).

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 multicentered studies organized by WHO have 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

MPA had little or no metabolic effects in relation to blood coagulation and fibrinolytic factors, platelet functioning, carbohydrate and lipid metabolism, liver, renal and thyroid function etc. (Whigham et al., 1979; Astedt et al: 1971).

All the synthetic progestins were found to decrease the circulating levels of high density lipo protein (HDL) cholesterol, which are one of the few metabolic changes that could be linked to an increase in the incidence of ischemic
cardiovascular disease (Miller et al., 1981). But MPA led to a decrease in HDL cholesterol (Kremer et al., 1980) and has less effect than other synthetic progestogens (Hirvoran et al., 1981). MPA raised fasting blood glucose and insulin levels and caused an increased response of both glucose and insulin to a glucose load. Though adrenocortical suppression was observed in cancer patients treated with massive doses of MPA (Hellman et al., 1976), no glucocorticoid effect and no change in the circadian rhythm of plasma cortisol were observed with contraceptive doses of MPA (Aedo et al., 1981).

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 bromosulphthelein retension (Amatayakul et al., 1980), while another showed some increase in the aminotransferases (Bajaj and Madan, 1983) and third group found no change in the aminotransferase but an impairment of hepatic changes of bromosulphthelein (Avari, 1990) as a result of competition by the circulating progestogen for hepatic excretory mechanisms.

Overall studies revealed that this MPA could be used in combination with androgen supplementation. Hence, interests have been developed for a combined contraceptive drug regimen based on long-acting progestogens and androgens. The Task Force is about to evaluate long-acting progestogens developed by WHO steroid synthesis programme for female applications for their potential for fertility suppression in men. All would require long-acting androgen supplementation (Waites, 1994).
DIHYDROTESTOSTERONE

Like all other androgens, testosterone is derived from the basic structure of androstane. All the androgens possess cyclopentano phenanthrene nucleus. Testosterone is the 17-β hydroxy, 3-one derivative of androgens. Testosterone in the male is produced by Leydig cells, from acetate indirectly and from cholesterol directly. Testosterone quantitatively the most important androgen synthesized in the organism is characterized by an oxo group in position 3, a hydroxy group in a 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.

The reduced form of testosterone is an active metabolite of androgen and is known as dihydrotestosterone (DHT). It is more potent than testosterone, as it is one of the non aromatizable androgens. By, esterification of this molecule at 17 position, its effects could be prolonged (Nieschlag and Behre, 1990). The structure of dihydro testosterone (DHT) is given in Figure 4.

EFFECTS OF DHT IN MALE REPRODUCTION

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 gonadotrophins and androgen levels more effectively than testosterone in rats implanted silastic elastomers containing DHT. Testicular
FIG. 4. DIHYDROTESTOSTERONE
(DHT)
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 plugs in cage mates. Moreover, accessory gland function was maintained while affected spermatogenesis was noted. DHT also induced complete knock off gonadotrophin 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 pituitary, 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 in motility but, sperm counts were not significantly affected in treated male rhesus monkeys, 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 midpiece, 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).
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).

Effects of dihydrotestosterone on hypothalamo-pituitary - testicular axis was studied by percutaneous administration of DHT to normal man. The results 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 plasma 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. Finally the potential long-term effects of high DHT levels on the prostate and anabolic effects need to be investigated (Nieschag and Behre, 1990).
In view of the ability of the testosterone to undergo aromatization to estrogen and the possibility of such metabolized estrogens to evoke unwanted side effects like gynecomastia and behavioural changes, this non-aromatizable androgen (DHT) is chosen with progestin, medroxyprogesterone acetate (MPA) to find out the contraceptive efficacy of this regimen in male rats.

SECTION II

PLANTS FOR MALE FERTILITY REGULATION

Research in the field of reproductive biology leads to the inescapable conclusion that human reproduction is simultaneously wonderous and imperfect. The almost endless series or delicate steps leading to conception require reproductive tract structures capable of carrying out highly specialised functions, precise timing and a meticulously programmed sequence of events (Priya, 1995). The drugs and plant products which have a reversible, post-testicular action on the normal function of sperm stored in the epididymis would have a number of major advantages over other methods that act by suppressing spermatogenesis. The effect of the drugs and plant products would be rapid in onset and, on cessation of treatment, normal sperm would return quickly in the ejaculate (WHO, 1992, 1994).

Numerous plants and their products have been screened for their antifertility effects in the laboratory animals and men. Nagarajan et al., (1982) have tested numerous indigenous plants having potential spermicidal activity in human beings and/or animals.
Figure 5.  (a) Showing a branch of *Balanites roxburghii* with fruits.  
(b) Fruit of *Balanites roxburghii* showing the pericarp region.
A. **Balanites roxburghii** (Fig. 5)

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

- **Gujarati**: Begarea
- **Hindi**: Hingan, Hingot
- **Marathi**: Hinganabet
- **Sanskrit**: Ingudi
- **Tamil**: Nanjunda

It is a spiny tree about 6 meters in height with glabrous branches ending in a very strong, sharp ascending spines (Kapadia, 1975). The internode between the thorn is about 1.5 cm. long. Thorns are strong, green with brownish tip 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 2.5 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 full 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). Chanim (1991) also reported the methods of vegetative propagation, flowering, fruiting, Hingota oil, oil from seeds, variation in diosgenin, bulk 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 Periplanata 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 Tripathi, 1991). In this study the most active compound was diosgenin - 3-O (alpha - L-rhamnopyranosyl (1-2) -
beta-D-glucopyranosidyl (1-3) beta-D-glucopyranosyl (1-4) - beta-D-glucopyranoside. Inhibiting approximately 73.63% feeding at 0.02% concentration. They were also assessed relative efficacy of all the tested compounds, using azadirachtin as standard.

Saponins from B. roxburghii was also investigated as a mosquito larvicide. LC 50 and LC 90 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 quinquefasciatus and Aedes aegypti.

Saponin extracted from fruit kernel was also tested against second and fourth instar larvae of three mosquito species, and LC 50 and LC 90 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 pulp 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 (Hoshio et al., 1992). Pericarp alcoholic extract of B. roxburghii has been demonstrated to have spermicidal
Figure 6  Showing whole plant of *Phyllanthus amarus*.
action on rat sperm in a recent study (Rao et al., unpublished data). The extract also possessed differential antifertility action in rats (Shah et al., 1994: 1995).

(B) *Phyllanthus amarus* (fig. 6)

The genus phyllanthus belongs to the family "Euphorbiaceae". Phyllanthus is a genus of herbs or underherbs chiefly distributed in the tropical and sub-tropical region of the world. About 24 species occur widely in India and some ornamental exotics are planted in gardens. The species for the study is *Phyllanthus niruri* Linn. or *Phyllanthus amarus* which is known in different languages as follows:

- Gujarati : Bhonyaanmali
- Hindi : Bhonyaabali, Jaramala, Bhuinanvalah
- Marathi : Bhulavali
- Sanskrit : Bahupala, Banupuspi, Bhumyamalaki
- Tamil : Kilanelli, Kilkaynelli
- Telugu : Nilaussirika, Nilavusari
- Urdu : Bhulamla
- Oriya : Bhulalo
- Malayalam : Kirganelli, Kizhanelli

The plant is common weed in gardens and in cultivated land. It is distributed throughout India, Ceylon, the tropics generally (except Australia). 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).

**Stem:** Often branched at the base, angular, leaf bearing branchlets, slender, spreading.

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

**Flowers:** Yellowish, very numerous, auxiliary, the males, 1-3, the females solifary. Sepals of male flowers 0.6 mm long, rounded, those of the female 1-2 mm long, oblong, subacute, with white margins, not enlarged in fruit, stamens 3, anthers sessile on a short column, styles minute, free 2-lobbed.

**Capsules:** 2.5 mm diameter, depressed globose, smooth, scarcely lobbed.

**Seeds:** Tri-gonous, rounded and with longitudinal regular parallel ribs on the back (Kirtikar and Basu, 1933).

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 dysentry. The fresh root is said to be an excellent remedy for jaundice. The powdered leaves and roots are pulverized and made into polutice 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 ounce rubbed up in a cup of milk is given morning
and evening or the root or the 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. In Konkan, the root rubbed down with rice water is given as a
remedy for menorrhagia. 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 the cure of intermittent fevers (Kirthikar and Basu,
1933). The fresh leaves are also considered to be a remedy for jaundice
(Sharma et al., 1979).

Extensive work has been done on Phyllanthus niruri, Linn. Ottow was the first
to isolate a bitter principle which he named Phyllanthin and assigned C_{39}H_{37}0_8 for its molecular formula. Later Pecklot examined the plant, but he could
not isolate any crystalline material. Krishnamurthy and Seshadri (1946) made
a significant contribution in the study of the plant by phyllanthin and
hypophyllanthin 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' -
butyrolignan with absolsute (8s, 8's) configuration (Row et al., 1966; 1967).
Another new compound viz., linteralin was also isolated (Ward et al., 1979).
Petrol extract of P. fraternus showed antifungal activity against
Helminthosporum sativum (Bhatnagar et al., 1961). The leaf extract showed
antifungal activity against Alternaria alternata (Bhowmic and Choudhary,
1982). In Clinical trial for hepatitis in 160 children, it showed no side effects
(Dixit and Achar. 1983).
An unusual ellagitannin from the biologically active polar fraction of *P. amarus* (Foo and Wong, 1992). 'Luk-tal-bal' used in their traditional medicine to treat jaundice, is a group of plants in the genus *Phyllanthus* (Bansiddhi, 1992). The enormous amount of effluents arising out of coal burning in Kashmir Power Plant Complex causes serious setback to the overall development of the drug plant *P. amarus* (Saheed et al., 1993). Phyllanthin and hypophyllanthin, lignan constituents isolated from *P. amarus*, when tested on various cancer cell lines did not show significant cytotoxic effect on any cell line. Biochemical effects of *P. amarus* was studied after oral administration of the drug to rats with respect to diabetes (Jhon and Krishnaswamy, 1993).

Alcoholic extracts of roots and leaves of *P. niruri* showed hepatoprotective effects in experimental rats (Agrawal et al., 1988). In a preliminary study, carriers of hepatitis B virus were treated with preparation of the whole plant root powder (Thyagarajan et al., 1988). Ellagic acid, gallic acid and geranin have been isolated from the ethanolic extract of *P. niruri*, and identified (Ueno et al., 1988). Studies on callus induction and growth in *P. amarus* and some related species as well as the inhibition of enzymes of hepatitis B and related viruses by callus extracts are described by Unander (1991). Recently, the extracts of the whole plant have exhibited spermicidal activity *in vitro* (Rao et al., unpublished data). The alcoholic extracts of it also reduced fertility rate in mice and rats (Shah et al., 1994; 1995).
In light of the above observations on these two plants, this study has been undertaken to elucidate the contraceptive effects of crude alcoholic extracts from unripe fruit pericarp of *B. roxburgii* and whole plant of *Phyllanthus amarus*, as a preliminary study.