CHAPTER I
INTRODUCTION

Our approach to development *per se* rests basically on the aim of securing welfare and prosperity for the people of the country. Development accrues from the judicious application of science or what may be called "the appropriate technology". From basic knowledge of science, many technologies may emerge. The applicability of each of the technology is specific and as such the most important factor is the proper choice of technology. Development is thus a multifactorial operation. It depends among other things such as the population, socioeconomic situations, demographic features, production and its cost, distribution, demand and supply and so on. The uncontrolled population growth, i.e., the population explosion is the major hurdle towards development, not only of our country, but also in the international scenario.

While in industrialized world, with a sound per capita income, has a population doubling time of more than 130 years, 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 world population is increasing at a rate of 90 million or more a year, of which about 19 percent around, is in India itself. Therefore, even if the present impressive growth rate is maintained in 90's and subsequent decade, more than 15 percent of the urban population and 25 percent of the rural population will still be below the poverty line in 2021. Quoting an exact number, the population of India reached at 901.21 million by the end of 1993 (Times of India, 26th April 1994). Thus the growing needs for medical services, food, fuel, shelter, environment,
education and employment will further create enormous pressure, imposing a growing uneasiness amongst scientists, technologists, social scientists, statesmen and even laymen.

The immediate and the most appropriate step against this "Monstrous evil" of population growth would be no doubt "the family planning". The need for 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. But these were slowly defeated, and today the human race is dragged towards a situation, which is certainly going to spell doom over him for which nobody, but human themselves are to be blamed.

The 1990's represent the critical decade for making progress towards a stable world population, and to provide them a socially, culturally and economically sound life. Only a committed combination of deep personal motivation and strong political will could provide the choice. Therefore the field is focused itself towards science in different ways and means to look forward to bringing in a momentary, if not a permanent relief from this unformidable calamity.

Thus, today research on Family Planning methods in human has given a high priority for improving the methods of birth control. Hence, the search for investigating fertility regulation methods is likely to come up with new ideas in the contraceptive technology field.

The initial research and studies in this field were carried out using female as an ideal model, as it was believed that the control of fertility in female was
easier than that of male. As the fertilization takes place inside the female, various techniques were implemented, with a view to prevent the release of the ovum, by hormonal preparation or the pill, by spermicidal creams and jellies, so that they could not provide the ideal environment for the union of male and female gametes thereby avoiding pregnancy.

However, now a days due to the elevated social status of woman in the society and due to the increased concern over the use of pills, surgical procedures and abortions, as methods of fertility control in female, the research has been channeled in other direction i.e., in males. Moreover, family planning is the planning of the birth of child desired by a husband and the wife. To prevent unplanned and frequently unwanted children, either or both the members of the couple can apply various methods of fertility regulation. Thus, 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 urgency of the need for male fertility control. It is equally important that the fertility control and infertility represent the two sides of a coin, i.e., that of fertility regulation, the researcher investigating the methods for male fertility regulation would likely to come up with the etiological factors responsible for male infertility which is still unknown.

This indicates the need of new, safe, reliable, convenient and reversible male contraceptive method which allows men to share more evenly the responsibility as well as the benefits of an effective family planning.

However, in spite of extensive efforts, it has not yet been possible to develop a safe reversible method of contraception for men. Any method for male
fertility control should fulfill certain requirements: i.e., 1) it should be effective
to a degree similar to existing female methods; 2) acceptable to both the partners;
3) rapid effectiveness; 4) showing no untoward side effects on progeny and 5)
fully reversible (Nieschlag et al., 1981).

EXISTING MALE CONTRACEPTIVE METHODS

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

Coitus interruptus and Periodical abstinence.

These are considered to be the common, easy methods of male
contraception. Coitus interruptus is one of the oldest known methods of birth
control and is practiced widely, especially in catholic countries. However, the
possible adverse psychologic 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. The average failure rate is also very high, i.e., about 10 - 15 /100 woman
years.

Condoms.

The condom, a coitally related method of contraception is an effective, non
systematic method of long range family planning that provides a safe means of
prolonging intercourse and premature ejaculation. In recent years, the
acceptability of the condom has been increased far fold due to the increased concern of AIDS and other sexually transmitted diseases. However, the condoms are primarily a temporary contraceptive method and the condoms have a limited shelf life, particularly in tropical countries. Moreover, the acceptance of condoms varies with personal attitude.

Vasectomy.

The earliest attempt to interfere with male reproductive system, was to prevent disease rather than conception. Interruption of vas deferens was thought by many to cure prostatic hypertrophy (Wolfers and Wolfers, 1974). In 1950 and 1960 voluntary sterilization became popular through vasectomy. Vasectomy may be accomplished by surgery or by the application of vas occlusive devices or some chemical occlusive substances. However, it has the disadvantage of being primarily a method of permanent sterilization, since the reunion of the severed portions of the vas deferens is usually not successful. However 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.

Effect of surgical vasectomy.

Even though, it is generally concurred that vasectomy is a relatively safe, reliable and in expensive means of contraception, the studies have raised questions about the possibilities of the post operative effects (Hafez, 1980). The changes occur in the testis as a result of occlusion of its outflow tract, have been
thoroughly investigated in laboratory animals. The reports range from marked degeneration of the germinal epithelium with an associated proliferation of testosterone secreting Leydig cells. More recently, Flickinger et al. (1993) reported severe inflammation of the epididymis after vasectomy due to the interstitial reaction.

The effect of vasectomy on the hormonal profile has been reported earlier. A significant elevations of LH and testosterone with a slight increase in FSH has been observed by Smith et al. (1976). Similarly, Whitby et al. (1976) found an elevation of LH in their study of 39 patients one year after surgery, whereas Skegg et al. (1976) found only a slight elevation of LH alone in their study. However, Korbrinski et al. (1976) found significant decrease in FSH and he attributed this to the existence of an unidentified independent testicular factor influencing FSH production. No changes in semen biochemistry and serum androgen levels were noticed by Chinoy et al. (1982) even after 1 to 20 years of vasectomy.

The presence of agglutinating antibodies were detected (Ansbacher et al., 1972) in the serum of vasectomized men. However, to date no well controlled study has supported this increased autoimmune phenomenon following vasectomy. In addition, a transient reduction in the ejaculatory volume has also been observed. More recently, some case control studies suggested that vasectomy may predispose to prostate cancer, where as other studies found no increase in risk (Guess, 1990). In addition, studies based on hospital cases suggested an association between vasectomy and the manifestation of a possibly pre-existing testicular tumor (WHO. 1992a).
Vas occlusive devices.

Similarly, the vas occlusive procedures which includes extra luminal devices and intraluminal devices were practiced. However in extraluminal devices a significant portion of the devices protrudes out side the vas deferens. Intraluminal devices implanted either with or without cannula include cylindrical plugs, spherical beads or threads of satire materials. The main disadvantages of these are lack of reliability, either in the occlusive, or restoration of the function when it is removed.

Moreover, it has been recognized for some time that two main factors limit the acceptability of vas occlusion; one is the necessity for a skin incision, which is unacceptable in some cultures; and the other is the lack of certain reversibility should the circumstances require. Amongst many attempts to develop simplified methods to overcome the limitation of vasectomy and vas occlusion, the research in China has led to two major technical improvements: The isolation and ligation of the vas deferens through a puncture (non-scalpel opening) in the skin and the development of technique for the percutaneous injections into the vasal lumen, of sclerosing or occluding agents through a hypodermic needle (WHO 1992a). Recent years, it has been established a method based on the percutaneous injection into the restricted portion of the vas deferens of liquid silicone to form plugs. (Zhao, 1990).

EXPERIMENTAL APPROACHES TO MALE CONTRACEPTION

The final objective in the field of research in male fertility regulation is to develop a safe, effective, reversible, and acceptable method as cited earlier. There are several vulnerable sites that could be used for male contraception; (1)
regulation of hypothalamic releasing hormones and/or gonadotropins, (2) spermatogenesis in the testis, (3) sperm maturation in the epididymis, (4) sperm transport in the vas deferens and (5) the biochemical characteristics of seminal plasma. However, there are several problems involved in this approach for practical male contraception; (1) difficulty in separating suppression of the major testicular functions like spermatogenesis and androgen production, (2) the long lag period from initiation of treatment until absence of sperm in the ejaculate, (3) the problem of reversibility and restoration of fertility, (4) the possibility that abnormal sperm may be initially produced, with subsequent production of a defective offspring, and (5) the lack of motivation of men to use a contraceptive, as the male is not the one who becomes pregnant.

Till now, the main approach for male contraception revolves around the suppression of gonadotropins and/or interference with their action.

**Antiandrogens**

Antiandrogens are compounds which prevent the expression of biological activity of androgens at target sites by inhibiting one or more of the following mechanisms, viz; (a) intracellular conversion of testosterone in to dihydrotosterone (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 (mRNA) from the nucleus, by binding to a ribonucleo protein. An inhibitory effect at one or more, these essential steps lead to a lack of expression of androgenic activity.
From earlier studies, it was realized that treatment with pure antiandrogens activate negative feedback mechanisms leading to an increased secretion of hypophyseal gonadotropins and thus elevated levels of circulating testosterone. Thus, the maintenance of antiandrogenic activity over an extended period of time was therefore not possible. Accordingly, these antiandrogens can not be used for fertility regulation (Schenck and Neumann, 1978; Neumann et al., 1978). However, antiandrogens such as cyproterone acetate (CPA) with additional progestational properties were considered to be suitable for male fertility regulation as secretion of gonadotropin may either remain unaffected or may even be inhibited following long term drug exposure.

It was suggested that, CPA acts at the epididymis and affects sperm maturation. Based on the hypothesis of higher threshold requirement of androgens for the maintenance of epididymal function, clinical studies were initiated in the hope that sperm maturation in the epididymis would be specifically affected, without the impairment of other androgenic functions (Moltz et al., 1980). However, the clinical studies (Koch et al., 1976; Roy et al., 1976) does not seem to support these results. In human, all the studies reported, indicate a definite inhibitory effect on spermatogenesis (Wang and Yeung, 1980). Cyproterone acetate, thus is believed to have both peripheral androgen antagonistic and antigonadotropic properties (Neumann et al., 1978; Bajag and Madan, 1983) and it is claimed to have interference with epididymal function in rat (Kaur et al., 1992). Studies with a combination of cyproterone acetate and testosterone were also carried out. However, this approach seems to be unfeasible as the antiandrogens are known to affect the adrenal function (Lee, 1983).
Drugs and plant products

A reversible, post testicular drug action on the normal function of sperm stored in the epididymis would be rapid in onset and on withdrawal of the drug, normal sperm would return quickly in the ejaculate. Thus, this approach would have certain advantage over other methods which acts on the hypothalmo-pituitary-testicular axis. There would be no disruption in normal endocrine function and the latent period required to suppress spermatogenesis would be avoided. Since the sperm spend only a relatively short time in the epididymis, any interference with their competence at this stage would be more likely to interfere with their motility, capacitation, and/or the acrosome reaction, the events specific to sperm.

Many chemical compounds with reversible effects on sperm stored in the epididymis have been described but all have been discarded because of their toxicity (Ray et al., 1991). Alpha-chlorohydrin and the 6-chloro-6-deoxy sugars were amongst the more interesting and best explored (Waites, 1993). A variety of other compounds, and their analogues such as sulphasalazines, imidazoles, pyrimethamine etc., are currently under investigation by various agencies (Rajalakshmi, 1985).

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).

Gossypol is a very well known dimeric sesquiterpene derived primarily from gossypium species (cotton seed) of the Malvaceae. Its antifertility effect is well documented and it has been tested in China in more than 8000 subjects.
(Diczfalusy, 1987). It acts on spermatid, spermatocytes, disrupts cell membranes and damages the sperm head (Prasad and Diczfalusy, 1983). Unfortunately gossypol is a toxic agent which causes hypokalemia (Qian, 1985). Moreover, gossypol appears to induce irreversible infertility in some cases (Prasad and Diczfalusy 1983). Hormonal and liver function changes have also been noted. Since, gossypol consists, equal parts of two enantiomers, of which only (-) gossypol is active in vivo (Maklin et al., 1985). There is a hope that the efficacy may be improved following the separation of the inactive, but toxic (+) enantiomer from (-) gossypol (Diczfalusy, 1986). Numerous other plants such as Tripterygium wilfordii (WHO 1990; Qian 1987) in China, Solanum xanthocarpum (Rao, 1987a), Terminalia bellirica (Rao, 1989), Abrus precatorius (Rao, 1987b), Vinca rosia (Stanley and Akbar, 1992), Carica papaya (Chinoy et al., 1984, 1994; Lohiya et al., 1992) were screened for their antifertility effects in India. However, the contraceptive efficacy varies from one plant to other. The work on the Tripterygium wilfordii would give some hope for the positive results (WHO, 1992a). It has been shown that a multiglycoside extract of this plant, caused reductions in sperm motility and concentration in male patients (Waites, 1993).

Immunological Approach

The various immunological approaches to fertility regulation involves the administration of an 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). The advantage of the latter approach includes immediate immunity with predictable and
adjustable dose. But the disadvantages are the short duration of the immunity, risk of sensitization and high cost. In view of these advantages, active immunization with a birth control vaccine is the generally favored approach. However, theoretical hazards include cross reactivity with non target antigens, formation of immune complexes, variable individual responses etc. The fertility regulating vaccines against hypothalamic, pituitary and gonadal hormones, as well as sperm, ovum, conceptuses and placental antigens are being explored.

Among hormonal antigens, studies were being conducted with LH-RH and its analogues, follicle stimulating hormones (FSH) luteinizing hormone (LH) and testosterone. However, with the exception of FSH, these approaches represent unattractive propositions because of the considerable risk of autoimmune damage and/or major endocrine damage (Moudgal and Rao, 1985). Among sperm antigens specific enzymes such as acrosin, hyaluronidase and the sperm specific lactic dehydrogenase (LDH-C₄) have been investigated. LDH-C₄ appears to be the most promising approach in this group, since a significant reduction in the fertility has been achieved in female baboons with a synthetic 15-amino acid peptide of LDH-C₄ (Mos - 15) conjugated with diphtherial toxoid (Goldberg et al., 1981).

Inhibin

It appears that normal tubular epithelium of seminiferous tubule produces one or more substances, that specifically control, the production of FSH through a negative feed back mechanisms. Thus, the inhibin with similar properties has been obtained from different sources, viz., rete testis (Setchell and Jacks, 1975) semen (Franchimont et al., 1975) and extract of testis (Lee et al., 1974). the
increase of interest for inhibin as a contraceptive stems from the observation that the administration of such substance might produce infertility without impairment of libido.

However, a decade of intensive research on inhibin has not produced a pure preparation of this protein. 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 function (Nieschlag et al., 1981; Sharpe et al., 1981). Work on specific inhibition of FSH with the testicular derived polypeptide inhibin continues (Sheth et al., 1985).

Passive immunization of adult rats, hamster and marmoset with anti-seminal inhibin resulted in complete or partial block of fertility (Sheth et al., 1992). Further it was shown that antibodies to human seminal plasma inhibin cause sperm agglutination and impairment of cervical mucus penetration and sperm egg attachment and also the spermatogenic inhibition (Mehta and Sheth, 1992).

HORMONAL APPROACH TO MALE FERTILITY REGULATION

Few advocates of this approach seem to realize that to develop a male contraceptive, "a pill for men" is a formidable task because, the spermatogenesis and the reproductive function in the male is a complex process which involves several series of delicate steps.

Hormonal factors play a key role in the control and regulation of reproductive function, each event of which is under the control of spontaneous, precisely timed hormonal interplay, resulting from the complex interactions between the hypothalamus, pituitary and gonads. The hypothalamic pituitary axis...
is basically the control center of reproductive function, through a series of neurohormonal secretions which cause the secretion of follicle stimulating hormone and luteinizing hormone. The gonads are the target organs which synthesize diverse steroid hormones. There is a dynamic relationship between the hypothalamic release of luteinizing hormone - releasing hormone (LH-RH) pituitary secretion of LH and FSH and testicular secretion of testosterone and inhibin. The pituitary production of gonadotropin and prolactin are regulated by the neurosecretory endocrine outflow of the hypothalamus (Reichlin, 1981). The pituitary hormones in turn regulate steroidogenesis and spermatogenesis in the gonads, which consequently exert a feedback regulation on the hypothalamus and pituitary (Debeljuk et al., 1972). The primary mechanism involved in the regulation of the GnRH - gonadotropin system depends on the negative feedback exerted by gonadal hormones (androgen and estrogen) and inhibin. Androgen primarily inhibits LH while inhibin affects FSH production (Robaire and Hermo, 1988). The finding that, the binding sites of FSH are on the Sertoli cells of seminiferous tubules, suggests its role in the secretion of androgen binding globulin and inhibin from these cells.

Data from animal studies suggest that FSH also induces appearance of LH receptors (Richard and Midgley 1976). Both FSH and LH acting sequentially are therefore required for spermatogenesis. The action of LH, possibly in synergism with prolactin (Bartke et al., 1978) stimulates the interstitial cells to secrete testosterone. The pulsatile secretion of gonadotropin release of LH from the pituitary gland which binds to specific cell membrane receptors of the interstitial cells of the testis, thereby initiating enhanced conversion of cholesterol to
testosterone is reported (Swerdloff et al., 1985).

**Organisation Of Spermatogenesis**

The spermatogenic process which takes place in the semeniferous tubule of the testis comprises of a series of events leading to the development of diploid spermtogonia into haploid spermatids. Spermatogonial differentiation has been thoroughly worked out mostly in rodents (Clermont, 1972). 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 in to spermatozoa (Fig.I). The stem cells are formed from the type A spermatogonia and they remain quiscent in the intact cells. These cells resume proliferation after damage occurs to the spermatogonial cells (Van Alphen et al., 1989).

**HORMONAL REGULATION OF SPERMATOGENESIS**

Testosterone is one essential factor for gametogenesis in males. The other hormone associated with spermatogenesis is follicle stimulating hormone (FSH) (Fig. II). FSH acts directly on the germinal epithelium (Means et al., 1976) while luteinizing hormone (LH) exerts its influence via testosterone produced by the Leydig cells. However, these functions are governed by the pituitary gonadotropin released in response to the hypothalamic gonadotropin releasing hormone (GnRH) (Plant, 1985).

**Testosterone and initiation of Spermatogenesis.**

Administration of androgen, LH or hCG in immature rats subjected to
A - SPERMATOGONIA

RESERVE RENEWING STEM CELLS STEM CELLS

PLOIDY

MITOSIS 2n

B - SPERMATOGONIA

PRIMARY SPERMATOCYTES

MEIOSIS I 4n

SECONDARY SPERMATOCYTES

MEIOSIS II 1n

ROUND SPERMATIDS

ELONGATED SPERMATIDS

Fig. 1
hypophysectomy or estrogen treatment did not start complete spermatogenic development. Germ cell formation did not pass beyond the meiotic stages of the appearance of early spermatid (Chemes et al., 1976; 1979; Chowdhury and Steinberger, 1975). In the study by Russell et al. (1987), LH only partially prevented germ cell loss induced by hypophysectomy. Thus, in the rat, testosterone alone is not sufficient for the initiation of the complete spermatogenesis. However, testosterone alone can initiate the development of elongated spermatid in primates (Chemes et al., 1982).

**Testosterone and maintenance of spermatogenesis**

Although, both FSH and testosterone have shown to be necessary for the initiation of spermatogenesis in most species, testosterone alone can maintain the fertility in the absence of FSH once the spermatogenesis has been established. In qualitative terms, all aspects of germ cell development were supported by testosterone. (Barlett et al., 1989; Buhl et al., 1982; Santulli et al., 1990). However, the quantitative maintenance of the spermatogenic process assessed from enumeration of germ cells in histological sections, was not fully achieved.

In the investigations by Sun et al. (1989) and Santulli et al (1990), it was noted that testosterone was unable to completely uphold the formation of testicular sperm. The effects of testosterone were dose dependent, but diminished with prolongation of treatment. However, in contrast to these observations, Robaire and Zirkin (1981) reported that testosterone alone maintained the daily sperm production over a 4 - weeks period in estradiol suppressed animals. Similarly DHT also effective in qualitative maintenance of spermatogenesis (Dube et al.,
Concomitant administration of testosterone maintained spermatogenesis in a quantitative manner over a period of 30 days in experimental animals treated with GnRH antagonists (Rea et al., 1986). However, this may be due to the testosterone supplementation which selectively stimulated pituitary and serum FSH concentrations. Subsequently, it was demonstrated that there is a positive feedback effect of testosterone on FSH in a testosterone dose-dependent fashion (Bhasin et al., 1988) and could also be induced with DHT (Arslan et al., 1989). However, in contrast to the rat model testosterone did not stimulate FSH in GnRH antagonist treated primates (Bagetell et al., 1989). Thus in conclusion it was reported that testosterone alone maintains spermatogenesis qualitatively but not quantitatively in rat and in primates.

The information about testosterone and maintenance of spermatogenesis in humans is based on trials with androgens for male contraception (Swerdloff et al., 1979; Nieschlag et al., 1989) and the studies of Matsumoto (1989), who used androgens combined with selective gonadotropin replacement for the study of hormonal regulation of spermatogenesis. In the latter, healthy volunteers received high dose treatment with hCG or testosterone for suppression of gonadotropin secretion. Despite the treatment of 9-16 months, azoospermia could not be achieved. It was concluded from these studies that testosterone alone can qualitatively maintain spermatogenesis in the absence of detectable FSH concentrations. This conclusion may not be entirely valid, since according to the recent data, the bioavailability of FSH was not totally abolished (Matsumoto and Bremner, 1990). These findings might also explain why azoospermia could not be induced consistently (60% of volunteers at maximum) in contraceptive trials with
testosterone. It must be pointed out however, that in the 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). This observations suggest that with respect to the development of an endocrine male contraceptive the type of androgen might be of importance.

**Testosterone and reinitiation of spermatogenesis**

In the hypophysectomised rat model, the ability of testosterone or LH to reinitiate or restore spermatogenesis was rather limited (Harris et al., 1977, Huang et al., 1987). Dihydrotestosterone was less effective than testosterone (Harris et al., 1977). However, Chowdhary and Steinberger (1975) reported a complete restoration of sperm counts following testosterone administration. Completely different results were obtained with other experimental approach i.e., by immunisation of LH and FSH and by estrogen treatments (Awoniyi et al., 1989a; 1989b).

Thus in conclusion, testosterone is not as effective in reinitiation as in maintaining the spermatogenic process in rats and primates (Weinbauer and Nieschlag 1990). Both testosterone and FSH are essential for reinitiation of the spermatogenesis in animals.

**Mechanism Of Testosterone Action On Spermatogenesis**

In spite of numerous and continued efforts, the molecular mechanisms and
precise events through which testosterone influences spermatogenesis still remain largely unknown. Most likely testosterone is converted to DHT within the seminiferous tubules before acting on spermatogenesis (Payne et al., 1973; Rivarola et al., 1973). Follicle stimulating hormone enhances the actions of testosterone eventually via an increase of Sertoli cell androgen binding protein (ABP) production resulting in an accumulation and increased availability of testosterone (Hansson et al., 1975). Conversely, however, testosterone increases the effects of FSH and stimulates the ABP production by itself. Stimulatory effects of testosterone on Sertoli cells mediated by peritubular cells have been documented (Skinner, 1987). Recently, it was found that both testosterone and FSH increased the Sertoli cell androgen receptors (Verhoeven and Cailleau, 1988).

Synthesis of RNA, in all stages of spermatogenesis was stimulated by testosterone alone and more so in combination with FSH (Parvinen and Soderstrom, 1976). More recently, Chandolia et al. (1990) and Weinbauer et al. (1989a) revealed that testosterone markedly influenced the effectiveness of FSH in the adult rat. It is speculated that testosterone regulates and enhances the responsiveness of Sertoli cells and spermatogenesis to FSH.

Hormonal Control Of Epididymal And Vas Deferens Function

The spermatozoa that leave the testis through the rete testis and efferent ductuli are not capable of fertilizing an ovum, until they have matured as they pass through the first part of the epididymal duct. The chemical mediators of this maturation process are still largely unknown. Specific epididymal proteins have been identified and purified from the caput epididymidis, but so far their functions
have not been fully established (Bajaj and Madan, 1983). Regulation of both the epithelial and lumenal functions is under complex hormonal control. Though androgens, and in particular the 5-α-reduced metabolite of testosterone, i.e., 5-α-dihydrotestosterone, are considered to be the primary modulators of epididymal functions. It has become apparent that many other regulatory molecules probably play specialized roles in maintaining normal functions within this tissue. Evidence has been gathered to indicate that for at least some specific facets of epididymal function, in addition to the endocrine regulation mediated by circulatory hormones, there is a paracrine regulation, i.e., regulation by factors entering the lumen of the epididymis (Robaire and Hermo, 1988).

Evidence provided by Jones and Glover (1973) indicated that, in the presence of androgen, the lining cells of the cauda epididymidis maintain a constant milieu in the lumen of the tubule as a result of their capacity for absorption and secretion. When androgen is withdrawn, as in bilateral gonadectomy, these functions are impaired and certain characteristic changes in the chemical composition of the lumenal fluid occur. During the second week of castration spermatozoa in the cauda epididymidis showed signs of disintegration and an increased process of decapitation (Glover, 1976).

Intensive research has investigated the endocrine milieu of the epididymis. 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 experimental animals, testosterone is the primary androgen in the seminiferous tubule, where as, DHT is the major androgen in the epididymal fluid. Direct evidence that dihydrotestosterone is pivotal for
spermatozoa to acquire their fertilizing potential has been obtained by assessing the effects of testosterone and dihydrotestosterone in the presence or absence of an inhibitor of 5-α-reductase activity in adult castrated male mice by Cohen et al., (1981). It was found that, the presence of an inhibitor of 5-α-reductase caused a decrease in the number of motile spermatozoa and the fertilizing capacity of the sperm.

There is a decrease in the concentration of androgens and androgen binding proteins (ABP) along the length of the epididymis (Hanson et al., 1974; Howards, 1983). In experimental animals, ABP is secreted into the seminiferous tubule by Sertoli cell under the influence of follicle-stimulating hormone (FSH) and flows in the epididymis, where it is thought to maintain a high concentration of DHT, which in turn is considered to be vital for sperm maturation. However, 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. It is thought, that the initial segment of the epididymis is dependent on intraluminal androgens and can not be maintained by exogenous androgens (Fawcett and Hoffer, 1977). Androgens and other larger molecule can not freely diffuse from the blood into the epididymal lumen because of a barrier to passive transport at specialized epithelial cell to cell tight junctions.

Brooks (1981 a,b) reported that, in the absence of androgens, epididymal intermediary metabolism is entirely dependent on carbohydrate for metabolic fuel. However, in the presence of androgen, primary metabolic fuel becomes lipids. This switch in dependence of different energy substrate is due to an increased lipid oxidation. The transport of ions across the epididymal epithelium is also
dependent on androgens.

The transport of spermatozoa, however, 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 luminal content down the excurrent duct system. In addition to the demonstrated actions of androgens on the excurrent duct system, there are a number of other hormones, e.g., estradiol, prolactin and vitamin 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).

**VARIous HORMONAL METHODS FOR FERTILITY REGULATION.**

The endocrine approach to male fertility regulation is based on the suppression of pituitary gonadotropin secretion. Since the selective inhibition of FSH may not result in azoospermia, which is considered as a prerequisite for male fertility control, FSH and LH have to be suppressed simultaneously, necessitating the need of testosterone substitution to maintain androgenicity (Nieschlag et al., 1985, 1989). Therefore, unless, it is possible to interfere selectively with spermatogenesis, with out affecting Leydig cell function or unless the substance used is androgenic by itself, the most methods require androgen substitution. The following endocrine approaches to male fertility regulation have been tested or are under consideration.

**GnRH analogues**

During recent years, synthetic peptides with closely similar structure to that of the gonadotropin releasing hormone appear to be a promising lead in male
fertility control (Waites, 1985). It was shown that natural and synthetic LH-RH not only stimulate the release of LH and FSH, but also augments the synthesis of these glycoprotein hormones in the anterior pituitary. LH-RH has been shown to have two major effects, either as potent LH-RH agonist or as LH-RH antagonists. On theoretical grounds, both agonist as well as the antagonist of LH-RH could be of use in the male fertility regulation. The antagonist would act at the level of anterior pituitary thereby interfering with the synthesis and release of both LH and FSH. Although, the prime aim of such an approach is to interfere with spermatogenesis by inhibiting the synthesis and release of FSH, it is obvious that a reduction in the level of LH would lower the level of circulating testosterone with likely the reduction in libido and secondary sex characteristics. Clinical studies to investigate the possible use of some of the LH-RH agnostic analogs for male fertility regulation have also been carried out (Waites, 1985; Nieschlag et al., 1985). LH-RH agonist was found to suppress the pituitary secretion of LH and FSH and of testosterone and to have a direct effect on the testis. However, an important consideration for such an approach is the negative feedback through testosterone, a decrease in its levels would enhance the endogenous synthesis and release of LH-RH from the hypothalamus. This would necessitate an increase in dosage of LH-RH antagonist if its therapeutic effectiveness is to be maintained. Moreover, toxic manifestations were also discovered with LH-RH analogues (Waites, 1985). Besides, the treatment with GnRH antagonist has the disadvantage that pituitary (Nieschlag et al., 1985) and testes are stimulated in the beginning of the treatment before the suppression is achieved. In addition, LH-RH antagonists of sufficient biological potency and proven safety are not available.
Similarly, 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 LHRH agonistic for male fertility regulation have also been studied (Waites, 1985; Neischlag et al., 1985). LH-RH agonist was found to suppress the pituitary secretion of LH and FSH and of testosterone and to have a direct effect on the testis. It has been shown that both sperm production and fertility recovered, after three years of continuous suppression to azoospermia induced by means of GnRH agonist. However, more data are needed regarding the efficacy, safety and side effects and acceptability of such an approach in the human male for fertility regulation. An inherent problem with the approach using LH-RH agonistic analogs for male fertility regulation is the reduction in circulating levels of testosterone. The reduced levels of testosterone over an extended period of time may interfere with libido and secondary sex characteristics, expressed through the biological activity of this hormone. The possible solution may be in utilizing a combined approach, where LH-RH analogs are used for the inhibition of spermatogenesis, while testosterone is administered exogenously to minimize the effect that would result from the lack of this hormone (WHO, 1992b).

**Sex Steroids**

Steroids are known to suppress spermatogenesis by inhibiting pituitary gonadotropin secretion resulting in sterility in animals and human beings. Thus, both LH and FSH can be suppressed by estrogens, progestins and androgens.
Estrogens

The effect of estrogens on male reproductive system are numerous (Johnson and Gomes, 1977; Rao and Chinoy, 1984; Rao et al., 1993; 1994a). The inhibitory effect of estrogens on testicular steroidogenesis is well documented (Dufau et al. 1978). These compounds have been used as a potent antispermatogenic and antifertility agents in males (Rao and Chinoy, 1983). It has been reported that, the estrogen treatment to mice manifested in maturational changes in epididymal spermatozoa as a result of androgen deprived effect (Rao and Mathur 1987). Similarly, many estrogenic compounds like diethylstilbestrol (DES) suppress spermatogenesis in man (Jackson and Jones, 1972). The treatment caused many abnormalities to the reproductive tract which included epididymal cyst with fibromuscular growth and metaplasia (Thomas et al., 1985). The beneficial role of androgen on the toxic effect induced by DES were reported by Rao et al. (1993a; 1994a). The androgen supplementation to the DES administered rats maintained the accessory gland function but, not the spermatogenesis. However, although estrogens are highly effective for their antifertility action, due to their adverse effects, they can hardly be considered to be the candidate for the role in male contraception. Even with the addition of androgens, feminizing symptoms such as gynecomastia prevails making the use of estrogens undesirable for the contraceptive use.

Progestins

Progestins have been used as antifertility agents, due to their antispermatogenic potential (Bennet, 1974). After being tested for their
antispermatogenic properties, they are classified into the following general categories.

i. Progesterone and its esters

ii. Progestagens (synthetic progestins) which include 19-nor testosterone derivatives.

iii. Antiandrogenic progestins.

Progesterone has been reported to be weakly antispermatogenic in many species (Ericsson et al. 1964, Ericsson and Dutt, 1965). These compounds were found to inhibit spermatogenesis poorly because of their weak antigonadotropic characteristics (Bennet, 1974). The weak antigonadotropic activity of these compounds may be further negated by their ability to act as substrate for testosterone synthesis.

The 17-acetoxy derivatives of progesterone are also poorly antispermatogenic agents. However, the results are variable and confusing. Medroxyprogesterone acetate was thought to inhibit spermatogenesis without altering libido in the human (Macleod, 1965). But unfortunately, the results were not forthcoming in the second trials (Patanelli, 1985).

Thus, the antispermatogenic properties of progestagens are exceedingly variable and dependent on the amount and the type of progestogen administered, its mode and period at administration and finally the species of the animal studied. Moreover, adverse side effects especially related to hepatic dysfunction were also observed (Bruce et al., 1977).

In general, administration of progestin in a dose sufficient to induce severe oligospermia or azoospermia also results in loss of libido and potency as well as
increased nipple pain. Furthermore, in order to produce the required degree of azoospermia or oligospermia higher amounts of progestins are required (Bajaj and Madan, 1983). Hence the application of a simultaneous androgen therapy would be preferred than progestins alone.

**Androgens**

Although, the inhibitory effects of androgens on spermatogenesis was demonstrated in laboratory animals almost forty years ago, their potential for male contraception has been extensively investigated mostly during the last decades. Though, the earlier reports on suppression of spermatogenesis by testosterone exits (Heller et al., 1950; 1958; Ludwig, 1950), the first positive test in humans using testosterone as a contraceptive agent was presented by Reddy and Rao (1972), who administered testosterone propionate by intramuscular injections resulted in azoospermia. Similarly, testosterone administered through sustained release capsules in rabbit (Ewing et al., 1973) and rats (Reddy and Prasad, 1973) produced azoospermia with out affecting libido. Due to its inherent drawback of daily injections, several clinical studies have used testosterone enanthate (a depot form) at varying dose and intervals (Paulsen and Leonard, 1976; Steinberger and Smith, 1977; Mc Clure et al., 1977). Even though, the results were not consistent, 200 mg of testosterone enanthate was found to cause a marked suppression of spermatogenesis. 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).
The inhibitory effects on spermatogenesis is completely reversible, with longest
duration of time to reach the pretreatment level was found to be 34-48 week
(Paulsen, 1980). Knuth et al. (1989a) reported that, even in body builders using
high doses of anabolic steroids for long periods, sperm production returned to
normal, when the androgen is discontinued. These properties of the androgen are
being made to use for the development of androgen, as a male contraceptive
(Cunningham et al., 1979; Knuth et al., 1989b, Matsumoto, 1988). However,
sperm production is not completely inhibited in all cases, even though the results
from recent studies appear to be encouraging (Matsumoto, 1990). This incomplete
effect may be due at least in part to the unfavorable kinetics of testosterone
enanthate. Clinical trials with testosterone preparations providing more even
testosterone serum levels in the physiological range are awaited with great interest.
An indication that kinetics closer to zero release order may be more effective, is
provided by studies using a 19-nor testosterone ester with longer half life than
testosterone enanthate (Knuth et al., 1985; Behre et al., 1990). Similarly a long
acting androgen (20 Aet -1) presently under trial may be a promising lead for male
contraception over other androgen esters (WHO, 1992a).

Moreover, due to the inability of testosterone alone in inducing
azoospermia, a condition thought to be essential for a contraceptive formulation
(Schaffenburg et al., 1981), numerous efforts have been made by combining
testosterone with other compounds such as estradiol, (Robaire et al., 1979)
progestagens and LH RH analogs. The results currently indicate a failure rate
better than condom use (WHO, 1992b).
The possible side effects of testosterone and its esters administration have been reviewed by Troen (1977) include weight gain, aggravation of acini and reduction in testicular size (Palaciose et al., 1981). The development of gynecomastia following androgen therapy is not completely understood. It may result from the peripheral conversion of androgen to estrogen, or conversion of androgen by the mammary tissue itself. Hence the levels of estrogen androgen ratio has to be monitored in these cases.

**Progestin - androgen combination**

Trials with progestin - androgen combination have been initiated with the hope that the adverse side effects (eg., impotence and loss of libido) which follow the long term use of progestin could be avoided. In addition, the dose of both the androgen and the progestin administered in combination may be less than that if either of these compounds are used alone for achieving male infertility. A variety of hormonal regimen have been tested for their ability to suppress spermatogenesis, sufficiently to act as an effective contraceptive (Knuth and Nieschlag, 1987; WHO 1992a; WHO 1993). The major objective of these clinical trials was to select the most optimal drug combination, which when administered through a generally acceptable route and the lowest effective dose level, which may result in a safe, effective and reversible method for male fertility regulation.

Of the various combinations tested so far, it seems that DMPA (200 mg/month) plus testosterone cypionate (250 mg/month) seems to be the most promising combinations (WHO, 1979). However, the most effective commercial combination of MPA + TE shows that, azoospermia has been achieved in only 50-
60% of the treated subjects, although oligospermia manifest in almost all the subjects. Azoospermia may not be persistently maintained even while the treatment is continued over an additional period of time.

**RATIONALE OF THE PRESENT WORK**

Due to the uncontrolled world population growth research on fertility regulation methods in human has been given a high priority. However, the development of methods for fertility regulation in male has been lagged well behind that of females. Despite intense research as mentioned above, no acceptable antifertility drug has been produced in male nor any drug regimen has yet been found to completely suppress the sperm in the ejaculate (azoospermia) of all men. Moreover, any acceptable antifertility pill for the male should be safe, capable of reversibly suppressing the sperm production or sperm function with out interfering with libido and potency or any other health status of men. The requirement of azoospermia could be relaxed, if it could be shown that the residual sperm from men whose sperm production are only partially suppressed (oligospermia) are incapable of fertilizing the ovum (WHO, 1992a). This strategy has pursued in the present study.

Since the spermatogenesis is under the control of hormones, the possibility of interfering this mechanism via endocrinological means appears to be a reasonable target. Thus hormonal suppression of spermatogenesis is currently being investigated as a reversible method of male contraception (WHO 1991). Exogenous steroids (androgen and progestin) are known to suppress the spermatogenesis by inhibiting the pituitary gonadotropin secretion (Bruce et al.,
A variety of hormonal regimens including androgen with or without progestin were tried throughout the world (Waites, 1988; Knuth et al., 1989b). 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 (WHO 1991; Knuth and Nieschlag, 1987). In addition, due to their synergetic action, the dose of both the androgen and progestin, when administered in combination will be less than that of these compounds, when used alone and thereby minimizing the side effects, if any.

The major objective of these clinical trials was to select the most optimal drug combination when administered through a generally acceptable route and lowest level which may result in the safe, effective and reversible method for male fertility regulation. However, the inability of these compounds to produce consistent azoospermia in all the treated individuals, raises the question regarding its efficacy as a male contraceptive (Matsumoto, 1988; Waites, 1993). Suppression of LH and FSH release with oral androgens and monthly or biweekly injections of testosterone does not produce azoospermia in all men. Pregnancies can occur in their partners, even for men with oligospermia during these regimens. Azoospermia is the only acceptable end point. Combination of injectable testosterone or with impeded androgen such as danazol, progestin such as medroxyprogesterone acetate or anti-androgens such as cyproterone acetate are also incompletely effective. Uniform reduction of spermatogenesis may not be possible in all men (Rabin et al., 1984).

The reason underlying this apparent nonsuppression of spermatogenesis in a minority of men is still unclear (Matsumoto, 1990). The possible explanations
include difference in the pharmacokinetics or sensitivity of the hypothalamic pituitary testicular axis to the exogenous sex steroids. However there are no differences in the total or bio-available androgens or the rate of degree of gonadotropin suppression between azoospermic and oligospermic responders during the first 4 months of steroid administration. Thus the heterogeneity in spermatogenic response to exogenous sex steroids can not be explained on the basis of differences in drug absorption, distribution or metabolism, nor can the differences be accounted for by differential sensitivity to the negative feedback inhibition of gonadotropin (Anderson and Wu, 1992).

These findings, now lead to raise to question whether complete azoospermia is necessary in male to achieve contraception. Previous studies in the experimental animals have suggested, that the sperm function and fertility may be compromised in animals with severely suppressed, but not absent spermatogenesis (Ewing, 1986; Robaire et al., 1984). Therefore, it is necessary to ascertain whether the sperm function is suppressed in oligozoospermic condition that brought about by these steroid treatments. Recent reports have shown that, the fertilizing capacity of the sperm assessed by hamsters oocyte penetration test (HOP) is markedly reduced in normal men, whose sperm production is severely suppressed by testosterone enanthate (TE) administration (Matsumoto, 1988), and it was nil in medroxyprogesterone acetate (MPA) + TE induced oligozoospermic men (Wu and Aitken, 1989). Also, it was noted an increased production of reactive oxygen species by the sperm as well as by the leukocytes in the semen of oligospermic men induced by MPA + TE. Moreover Rajalakshmi et al. (1990) have also reported morphological and ultrastructural changes and plasma
membrane alterations in residual sperm of DHT treated rhesus monkeys. The progestins with and without androgen induced degenerative changes in the late spermatids, biochemical and morphological changes in the testis, epididymis as well as sperm in rat (Bhiwgade et al., 1991; Avari et al., 1992; Rao, 1991, 1992). Studies such as these are reassuring, but information is scanty in order to assess the causative factors for the loss of sperm function and the contraceptive efficacy of these steroids.

If the study could demonstrate that the contraceptive efficacy is high even when the spermatogenesis is not fully suppressed, the goal of developing a male hormonal pill or antifertility agent would be greatly simplified (Waites, 1993). Hence, in the present study, a combination of androgen (TE) and a progestin (MPA) has been used to evaluate their contraceptive efficacy in relation to the residual sperm function. The adverse effects of these treatments on the vital organs as well as the blood and clinical chemistry parameters were evaluated. The complete recovery status of these treatments were also assessed. Parallel set of treatment with MPA were also run along with this group in order to give a clear picture of the possible adverse effects caused by the progestogen (MPA) alone and the extent to which these were compensated by the TE in the combination group (MPA + TE).

MEDROXYPROGESTERONE ACETATE

Depot medroxyprogesterone acetate (DMPA, Depo Provera) is not only, but certainly the best known type of injectable contraceptive drug, due to its efficacy
FIG A. MEDROXYPROGESTERONE ACETATE (MPA)
and safety which have been reportedly demonstrated. It is an injection of long acting depot medroxyprogesterone acetate (DMPA), which is a hormone of the progesterone type formulated so as to release slowly from the site of injection. It is one of the most widely studied hormonal contraceptive drug. A review of numerous publications, which recorded studies of this progestational contraceptive formulation have given MPA a leading role in the contraceptive trials (Fraser, 1981, 1982; Benagiano, 1977; Mishell, 1976; Fraser and Holck, 1983).

MPA was first synthesized in 1958 (Babcock et al., 1958). It is a 6-methyl progestin, also known as a potent progestational steroid which possess antiandrogenic, syndrogenic and glucocorticoid activities (Brown et al., 1979; Lin et al., 1978; Bullock et al., 1978).

Chemistry and Pharmacology

MPA belongs to the class of C-21, 17-α-acetoxyprogestogens and is similar in structure to natural progesterone. It has the formula 17-acetoxy 6-methyl-preg-4-ene 3, 20 dione (Fig A). The unusual stereochemistry of the crystal structure (Duax et al., 1978) seemed to be important for its slow release into the blood. It has also been suggested, that the formulation procedure and the size of the microcrystals were critical for optimal prolongation of the duration of action (WHO, 1982).

MPA is rapidly metabolized with a metabolic clearance rate of 1,668 L./24 hours and has a plasma half life of about 4 - 5 hours (Besch et al., 1966). The levels in the plasma fluctuate from day to day, but fall steadily over the next few months. The microcrystals of MPA have a very slow solubility in the body fluids.
and that provides the prolonged action (Mishell, 1976). However an initial high release occurs into the surrounding tissues (Mishell et al., 1977).

The peak values of MPA were found 2 days after the injections, similar to testosterone, indicating that the bulk of both these compounds reach the bloodstream rapidly. However, the levels of MPA remained relatively high, 18-20 weeks after the last injections, indicating a slow elimination rate (Hedman et al., 1988). A slow elimination rate of MPA also has been demonstrated by Lan et al. (1984), suggesting that the kinetics of DMPA metabolism are similar in men and women.

Biological effect of MPA

DMPA is registered as a contraceptive agent in at least 83 developed and developing countries. In USA the Food and Drug Administration (FDA) clearance for the contraceptive use of DMPA was not granted for almost 25 years, because of the fear that it may increase the incidence of breast cancer. This fear stemmed from the observation that the treatment of beagle dog with 25 times, the human dose of Depo-provera resulted in the development of breast nodules. However, the subsequent reports showed that the studies carried out in beagle dog were not predictive of any cancer risk in human (Fraser and Wiesberg, 1981). Similarly, epidemiological studies of Greenspan et al. (1980) also showed no increase risk of breast cancer in woman after DMPA treatment. Thus, WHO have declared Depo-provera to be an effective method of contraception (WHO 1982). The FDA approval for the use of MPA was granted in USA in October, 1992.

The use of steroidal methods of contraception is contraindicated with the
history of cardio-vascular disease, liver disease, cerebro-vascular disease and reproductive cancer. Thus the side effects of MPA are similar to those seen with other contraceptive methods (Mosse and Heaton, 1990). However other reports suggest that MPA had little or no effect on these functions (Whighham et al., 1979; Astedt et al., 1971). Contrarily, Lee (1981) reported that the untoward side effects of MPA include Cushingoid features, excessive weight gain, adrenal suppression and chromosomal damage in a long term follow up study of 15 girls who were treated with MPA for central precocious puberty. Similarly, adrenocorticoid suppression was observed in cancer patients treated with DMPA (Hellman et al. 1976). However, no glucocorticoid effect was observed with the contraceptive doses (Aedo et al. 1981).

The effects of DMPA on liver function seem to be conflicting. Amatayakul et al. (1980) reported that DMPA produced no significant changes in any of the liver enzymes studied, whereas, the studies of Aldercreutz and Tenhuman (1970) showed an increased aminotransferase activity. Similarly, Saleh and Abd-El-Hay (1977) reported a change in hepatic bromosulphthalein.

The effects of DMPA on lipid metabolism evoked a number of responses. Kremmer et al. (1980) and Miettingen et al. (1981) reported a decrease in HDL cholesterol after DMPA administration, whereas Hirovoran et al. (1981) reported that DMPA had less effect in these parameters than other progestins. A reduction in plasma HDL cholesterol, induced probably by the DMPA administration was also reported by Hedman et al. (1988) and Friedl et al. (1985). Contrary to this, Fraser (1983) did not find any effect of DMPA on fasting triglyceride levels or on its composition. However, all the synthetic progestin were found to decrease
circulating levels of high density lipo protein (HDL) cholesterol and these changes could be linked to an increased risk of ischemic cardiovascular disease (Miller, 1981) and concern has been expressed regarding the effect of progestagens on lipid metabolism and transport (Fraser, 1983; Stadel, 1981).

Though, progestagens are known to affect carbohydrate metabolism, several studies reported that DMPA had little or no effect on carbohydrate metabolism (Amatayakul, 1979; Beck et al., 1977). However, Fraser (1981) and Vermeulen and Thiery (1976) in their studies reported that the DMPA raised the fasting blood glucose levels and insulin levels and caused an increased response to a glucose load.

In general, the contraceptive dose of DMPA were not found to have any significant effect on liver, kidney, thyroid or hemostatic function (Amatayakul, 1979).

Effect of DMPA on male reproductive function

Medroxyprogesterone acetate was thought to inhibit spermatogenesis in humans without altering the libido (Frick et al., 1976, 1977; Sanchez et al., 1977). A weak antispermatogenic effect of these compounds were observed in rat and monkeys (Bennet, 1974). Administration of large doses of DMPA to rats, rams or normal men resulted in azoospermia. In rat, MPA has been reported to reduce spermatogenesis indirectly by inhibiting gonadotropin secretion (Flickinger, 1977). In addition several studies suggest that MPA has a direct effect on Leydig cells (Satyaswaroop and Gurpide, 1978; Worgul et al., 1979; Barbieri and Ryan, 1980; Rao et al., 1994b). DMPA decreased the circulating gonadotropin (Barbieri and
Ryan, 1980) which in turn lowered the circulating testosterone, thereby causing its effects (Lobl et al., 1983). In normal men, serum concentration of LH and FSH and testosterone registered a fall in their values after treatment with MPA (Mayer et al., 1977; Melo and Coutinho, 1977). A fall in the plasma testosterone as result of the decrease in the gonadotropin levels was also recorded (Altman et al. 1972). The antiandrogenic effect of MPA might be due to its ability to suppress the circulating testosterone either by:

1. an increased metabolic clearance rate of testosterone,
2. by decreasing circulating gonadotropins,
3. by directly interfering with the Leydig cell function.

An induced hepatic steroid A-ring reductase activity was observed in rat and human by MPA (Altman et al. 1972). This increased hepatic enzyme activity could be correlated with a significant increase in the rate of irreversible removal of testosterone from the plasma as measured by the metabolic clearance rate of this steroid (Gordon et al, 1970). Similarly, a decrease in the production rate as well as tissue uptake of testosterone was observed after DMPA administration (Nolten et al. 1976; Clark et al. 1970; Albin et al., 1973).

The direct effect of DMPA on Leydig cell steroidogenesis in adult rat testis could also be due to the suppression of 17 - β - dehydroxy steroid dehydrogenase activity (Satyaswaroop and Gurpide, 1978). Similarly, in vitro studies of Barberi and Ryan (1980) showed an inhibition of testosterone production in rat interstitial cells. The rat testicular steroidogenic enzymes were also inhibited in the above study as well as in the studies of Rao et al. (1994b). Sunde et al. (1982) demonstrated that DMPA was a potent inhibitor of 3 - α -hydroxy steroid
oxidoreductase activity in the rat testis, epididymis, kidney and adrenal glands.

Although it was reported that MPA and other progestin alter the normal release of ABP from the Sertoli cells, it was Lobl et al. (1983) who suggested that during MPA treatment, the testicular and epididymal ABP content declined in parallel with the organ weights, serum levels of LH, FSH and testosterone, in contrary to an increased serum ABP concentration. This suggests that MPA acts directly on the Sertoli cells resulting in an increased ABP release into the blood.

The report on selective binding of gestagens on human sperm (Cheng et al., 1981 and their inhibitory effect on sperm motility in vitro (Kesserü et al., 1975; Hyne et al., 1978; Cheng and Boettcher, 1979) suggested a possible direct influence of MPA on sperm motility. However, Knuth et al. (1989b) suggested that MPA acts on sperm motion characteristics via an altered function of the epididymis.

The major hesitation to the contraceptive use of the progestin alone appears to be whether, the subsequent reduction in blood levels of testosterone, which may lead to anabolic imbalance (de la Torre et al., 1979) and/or sexual disturbances. Hence, a combination of androgen with DMPA or other progestin is essential for male contraception and needs to be substantiated with further studies (Hedman et al., 1988). Similarly, long follow up periods are required to provide definite evidence that prolonged suppression of gonadotropin would not persistently impair the gonadal function (Mauss et al., 1978).

TESTOSTERONE ENANTHATE

As all other androgens, testosterone derives from the basic structure of
FIG B. TESTOSTERONE ENANTHATE (TE)
androstane. This molecule consists of three cyclohexane and one cyclopentane ring (perhydrocyclopentane-phenanthrene ring) and a methyl group each in position 10 and 13. Testosterone, the quantitatively most important androgen synthesized in the organism is characterized by an oxo group in position 3, a hydroxy group in a position 17 and double bond in position 4 (Fig B).

To make testosterone therapeutically effective, three approaches have been used;
1. different routes of administration,
2. esterification in position 17, and
3. chemical modification of the molecules.

Thus esterification of the testosterone molecule at position 17, with a propionic or enanthic acid, prolongs the activity of testosterone in proportion to the length of the side chain when administered intramuscularly (Junkman, 1957). Several studies showed that after intramuscular administration, the androgen ester is slowly absorbed into the general circulation and then rapidly converted to the active unesterified metabolite (Fujioka et al., 1986).

Pharmacokinetics of testosterone enanthate.

It is known from the clinical studies for male contraception that testosterone or 19-nortestosterone esters suppress the endogenous LH and testosterone secretion (Nieschlag et al., 1989). In normal volunteers, the testosterone measurable in the serum is the sum concentration resulting from the endogenous testosterone and the serum concentration of the exogenous testosterone hydrolysed from the ester. Because, the endogenous testosterone is suppressed to hypogonadal
values during the first days after administration of the androgen ester, the changes in serum testosterone concentration after administration represent the combined pharmacokinetics of endogenous and exogenous testosterone.

The free unesterified testosterone has a half life period of only 10 minutes and would have to be injected very frequently for testosterone substitution or contraceptive trials, for which the testosterone esters with a prolonged half life are widely used as intramuscular injections (Nieschlag and Behre, 1990). As the length of the ester moiety determines the duration of the action, the pharmacokinetics of the different esters varies. Thus for substitution purposes, testosterone propionate must be injected every 2-3 days while testosterone enanthate allows a spacing regimen of 2 weeks. Injecting 250 mg of testosterone enanthate every 2 weeks, results in maximal supra physiological testosterone serum concentration, observed shortly after the injection (8 hours) and lower ranges were observed shortly before the next injection. If the injection intervals is extended to three weeks, 14 days after the injection, the serum concentration below normal range were observed. Two, other clinically available testosterone esters, testosterone cypionate and testosterone cyclohexanecarboxylate have very similar kinetic properties of testosterone enanthate (Nieschlag and Behre, 1990; Gooren, 1987; Schurmeyer and Nieschlag, 1984).

The disadvantage of all these esters is that, they produce initially supra physiological testosterone levels which may exceed normal levels several fold, and then slowly decrease, so that before the next injection pathologically low levels may be reached. The patient recognizes these ups and downs of testosterone levels in parallel variation of the general well being, sexual activity and emotional
stability. Because of these short comings, the World Health Organization (WHO) initiated a steroid synthesis program (Crabbe et al., 1980). Out of which a specific ester namely testosterone trans-4-n-butylcyclo-hexylcarboxylate (20 Aet-1) when tested in castrated monkeys found to raise testosterone serum levels of the animal in to normal range for about 4 months when a single dose of 40 mg was injected. The same amount of testosterone given as testosterone enanthate, produced supra physiological serum testosterone levels for 8 days, which returned to the subnormal range by 3 weeks (Weinbauer et al., 1986; Rajalakshmi and Ramakrishnan, 1989). Thus, 20 Aet-1 shows a kinetic profile that makes it a desirable entity for clinical use, in the treatment of hypogonadism as well as for substitution in hormonal male contraception. In a preclinical study in monkeys, 20 Aet-1 in combination with GN-RH antagonist proved very successful in achieving azoospermia (Weinbauer et al., 1989b). Currently first WHO sponsored studies in humans are being performed and the results are awaited with great interest.

Testosterone enanthate as a male contraceptive

The first efficacy study in hormonal male contraception sponsored by WHO (WHO, 1990; Waites, 1988), showed that testosterone enanthate is a contraceptive once the azoospermia has been achieved. But only two third of enrolled men developed azoospermia. Thus, the interests of those working on male contraception, converged in the attempt to develop a testosterone preparation providing serum testosterone levels in or close to the physiological range after administration.
Effect of Testosterone on the testes

Though, the testosterone is necessary for the initiation, maintenance, reinitiation of spermatogenesis, a dual effect which led to the suppression of spermatogenesis was also observed with testosterone in males of several species (Ludwig, 1950, Ewing et al, 1973). Small doses of testosterone cause atrophy of the seminiferous tubule (Ludwig 1950). The ability of testosterone to induce azoospermia was species specific (Ewing and Robaire, 1978). In rats, monkey and men testosterone treatment resulted in oligospermia (Reddy and Prasad 1973; Steinberger et al., 1978; Robarie et al., 1979; Lobl et al., 1983). Administration of testosterone enanthate is also found to suppress the testicular spermatogenesis without any adverse side effects on accessory sex gland function in rats (Rao et al., 1994c).

Similarly, high doses of testosterone esters decrease the levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH), as does the response to luteinising hormone releasing hormone (LH-RH). Testicular volume decreased (Palacios et al., 1981) and sperm production was found to be diminished by 90% or more in a dose dependant fashion. Volume of the ejaculate is usually unchanged (Cunningham et al., 1979; Palacios et al., 1981; Mauss et al., 1978). These effects appear to be reversible, and stemmed from the observation that even in the body builders using high doses of anabolic steroids for long periods, the sperm production may return to normal when the androgen use is discontinued.
METABOLIC EFFECTS

Haemopoietic System

The effect of androgen treatment on erythropoiesis have been well established and have been used in the treatment of a variety of anemia (Hendler et al., 1974; Hengstum et al., 1979; Najean 1981). When normal men are treated with pharmacological doses of testosterone esters, haemoglobin increase is about 1g/dl (Cunningham et al., 1979). Animal experiments offer some evidence that androgen enhance platelet production in the bone marrow (Rosenblum et al., 1987) and also promote the platelet aggregation (Johhson et al., 1977).

Carbohydrate metabolism

Though earlier studies suggest that androgen had no effect on carbohydrate metabolism, there is some evidence that androgens may influence carbohydrate metabolism (WHO, 1992b). It has been established that, there is a link between hyper androgenism and insulin resistance (Chang et al., 1983). However, the nature of this theory is still unclear. It may probably due to an interference with insulin receptor interaction, by increasing liver gluconeogenesis (Souba et al., 1988) or by promoting pancreatic insulin secretion (Chang et al., 1983). Many have reported a glucose intolerance or insulin resistance after the treatment with anabolic steroids (Godsland et al., 1986: Cohen and Hickman 1987). The data from our laboratory also suggest that administration of testosterone enanthate alters carbohydrate metabolism (Rao et al., 1994c).

Kidney Function

Apart from stimulating kidney growth, protein synthesis and erythropoietin
secretion, androgen does not appear to have any major effect on kidney (Mills et al., 1979; Gooren and Polderman, 1990; Rao et al., 1994c)

Liver function

Androgens can impair liver functions as evidenced by consistent elevation of various liver enzymes and some plasma proteins. These may include blood clotting factors II, V, VII, plasminogen and haptoglobin. Blood levels of fibrinogen, transferrin, sex hormone binding globulin, the T\textsubscript{4} binding globulin and cortico steroid binding globulin may decrease in the course of the treatment (Mooradian et al., 1987; Gooren and Polderman, 1990). Some serious effects on the liver due to androgen treatment are development of blood filled cysts in the liver, angiosarcoma and hepatocellular carcinoma (Carrasco et al., 1984; Aronold and Kaplan, 1979). There is some evidence that testosterone may act as a tumor promoter after initiation by other cause (Schulte et al., 1983).

Thus, the safety of long term use of testosterone in hypogonadal men is well proven, and the benefits of androgen replacement out weigh its risks. However for other potential uses of androgen therapy such as in male contraception, the risk benefit ratio must be evaluated in a manner similar to that used for estrogens and progestin for contraception in females.

In view of these considerations, the present study has been undertaken to assess the contraceptive efficacy of steroid combinations in relation to the residual sperm function in adult male rats.