CHAPTER I

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HISTORY OF CONTRACEPTION

For thousands of years couples have tried to control their fertility using various methods and techniques. The earliest mention is in the Bible, in Genesis Chapter 38, where Onan was reported to practice ‘coitus interruptus’ or withdrawal, ‘spilling his seed’ on the ground. This method is still used today.

Papyrus texts dating back to 1850 BC said upper class women put various substances in their vaginas to block or kill sperm. These included crocodile dung pessaries, different gums and a mixture of honey and sodium carbonate.

One out of four U.S. children are born out of wedlock, while 1.6 million U.S. women abort and decline parenthood each year.

Recent data from the United Nations indicate that men’s contribution to contraceptive use worldwide is only 15% whereas women’s contribution is 43%. The world’s population now at 5.8 billion – has doubled since 1957 and continues to add about 80 million people each year. Some 43% of men questioned believed that they should share the responsibility for contraception. Men and women are equal partners in conception, but not contraception – an inequity illustrated by a stark statistics: 80 percent of the world contraceptive users are females.

A World Health Organization (WHO) study in men found that between 41 and 75 percent of men would welcome a safe, reversible, convenient non-surgical male contraceptive which could be used separately from intercourse (WHO, 1982a). For women choices available are the diaphragm, the sponge, IUDs, the pill, cervical caps,
“morning after” pills, Norplant, Depo Provera, natural methods, ovulation detectors, the female condom, foams, jellies, suppositories, sterilization and more (Hatcher et al., 1990).

In the world, more than 500 million couples want to limit their fertility, temporarily or permanently, but are unable to do so. Very few options for men to initiate fertility control are currently available. Contraceptive needs of couples vary according to their type of relationship, purpose of contraception and age. Currently available methods of fertility regulation for men and women do not adequately meet the varied and changing personal needs of couple in their reproductive lives and in the widely different geographical, cultural, religious and service delivery settings around the world.

In industrialized countries many men seek to limit their fertility until children are desired, which can often be more than ten years after sexual maturity (Gross, 1993).

NON-HORMONAL METHODS

ABSTINENCE

Abstinence from intercourse is not necessarily sexual inactivity per se. Outer course is probably a better term to describe sexual activity other than peno-vaginal penetration.

When combined with the fertility awareness method, also known as periodic abstinence, or Billing’s method, its efficacy can be increased.
WITHDRAWAL

The withdrawal of the penis prior to ejaculation is very user-dependent and therefore unreliable. Even when performed perfectly, sperm can be present in pre-ejaculatory secretions and result in pregnancy.

CONDOM

The male condom is a thin tube of soft rubber that is rolled onto the erect penis and prevents sperm from entering the woman’s cervix. Protection from HIV and other STDs is a critical health concern, and condoms are the only widely available product that offers dual protection, so several condom manufacturers, as well as the public sector are investigating new material and designs for male condoms. Minor changes in condoms, such as the material from which they are made or their size and shape, could lead to greater acceptability and thus greater use of this important and low cost method. Condoms are also now available for a female.

VAS BASED METHODS

Vas based methods rely on cutting, blocking or otherwise limiting fertility in the vas deferens, the passage through which sperm travel from the epididymis to penis.

VASECTOMY

Male sterilization or vasectomy is the most effective of male methods of contraception currently available. Expanding use of vasectomy, as a safe and effective method requires overcoming several obstacles, namely socio-cultural and political barriers, provider bias, and inadequate information and communication. Currently
research needs are related to increasing information about the safety and efficacy of the procedure, dispelling myths and increasing male acceptance of sterilization. Greater acceptance of vasectomy is affected by two main factors: (I) the need for a surgical intervention; (II) the fact that the procedure needs to be considered permanent, as the success rate of reversal is low.

**NO-SCALPEL VASECTOMY**

No-scalpel vasectomy, originally developed in China, has been introduced in twenty countries (Martinez-Manautou et al., 1991) and is gaining worldwide recognition. After application of local anaesthesia, a specially designed vas fixing forceps encircles and firmly secures the vas without penetrating the skin. A curved hemostat with sharpened points is used to puncture the skin and vas sheath and stretch a small opening in the scrotum. The vas is lifted out and occluded as in other vasectomy techniques (Huber, 1989). No-scalpel vasectomy is ultimately faster and safer than traditional vasectomy (Nirapathpongporn et al., 1990; Li et al., 1991).

**PERCUTANEOUS INJECTION**

This method is completely non-surgical. It is thus less threatening and less risky than even the no-scalpel method (Goldsmith et al., 1985). The vas deferens is first secured and kept from moving around under the scrotal skin by the placement of a gentle clamp, which encircles the section of vas and skin. Once the vas is secured, a puncture needle is inserted into the vas and then replaced by an injection needle. Two tests are
performed to make sure that the needle is correctly placed and then the fertility limiting substance is injected (Shunquiang and Jinbo, 1986).

This method has the potential to be much more widely accepted than vasectomy in cultures with religious proscriptions against skin incisions. It is a delicate procedure, requiring training and precision and must be done exactly right.

Over 600,000 percutaneous injections have been performed in China (Liu and Li, 1993), the majority with sclerosing chemicals as the injectable. Some other options currently being studied around the world are neem oil injections (Upadhyay et al., 1993) and injections of tiny amounts of controlled release gossypol (Ye et al., 1993).

ADVANCES IN FERTILITY LIMITATION INSIDE THE VAS

There are four major new approaches to limiting fertility once inside the vas:

(i) **permanent chemical injection**:

A number of substances can cause permanent sterilization by injection. Chinese researchers have performed vas-based chemical sterilization with a combination of carbolic acid and n-butyl cyanoacrylate in over 500,000 men with resultant azoospermia in 96% of cases (Xiao, 1987). A World Health Organization (WHO) test inspired by this study resulted in 90% effectiveness in the 900 men when followed up at 9-12 months. Whether closing the vas deferens or other vessels, a sclerosing approach generally leads to a certain number of failures (Chvapil et al., 1990). The scar tissue is not solid and compact, allowing occasional recanalization.
(ii) Injectable Plugs

Injectable plugs are designed for one-time reversibility. They are ideal for men who believe they want permanent sterilization but would like the possibility of reversal in case of death of a child or some other unforeseen circumstance. In China over 512,000 men have received injectable plugs, which involve the same procedure as chemical injection, except that instead of a sclerosing chemical, polyurethane or silicone is injected and hardens to form a plug. In 1991, the WHO started trials in ten men with plugs made of silicone providing azoospermia in all men within 5-9 months (Zhao, 1992).

(iii) The Shug

The “Shug” (short for silicone plug) is a non-injectable plug alternative. Its main advantage over injectable plug is its double design, which gives it the potential to be more leak-free. Composed of two silicone plugs with nylon tails to help anchor the plugs to the vas, the shug can be inserted into the vas deferens using the no-scalpel method. After researchers found about a 97 per cent decrease in motile sperm count in the men studied, they subsequently decided that a properly sized shug would be 2-4 times larger than the one’s they used and repeated trials with larger softer devices. Of the six men in this trial, four men showed zero sperm counts a month after implantation and two men showed only a few sperm, all dead (Zaneveld et al., 1990). Further trials continue to refine the device.

(iv) S.M.A.

This method uses the polymer styrene maleic anhydride (SMA) with either the percentaneous or no-scalpel technique. When injected into the vas deferens, this polymer lowers the pH of the vas deferens enough to kill sperm passing through. This method is
occlusive and keeps the vas in an essentially undamaged and natural state. Since the polymer remains primarily whole, it can be flushed out at any time by an injection of dimethyl sulfoxide (DMSO), a bioacceptable solvent in the small quantities necessary (Rubin, 1983).

HEAT METHODS

Heat methods are now being “discovered” as the newest method of male contraception. These methods derive their effectiveness from the simple fact that the testes must be several degree cooler than the normal body temperature in order to maintain proper spermatogenesis (Rock and Robinson, 1965).

SIMPLE WET HEAT

This method is in the form of hot water which is inexpensive and available to everyone, was the first contraceptive heat method discovered by the scientific community.

Voegeli’s program for temporary sterilization is as follows: A man sits in a shallow or testes-only bath of 116°F for forty-five minutes daily for three weeks. Six months of sterility results after which normal fertility returns. Her attempts to publicize it were generally unsuccessful, although in 1954, the Japanese government requested the information and conducted several successful experiments.
ARTIFICIAL CRYPTORCHIDISM

Dr. Roger Mieusset of France, currently the Chief proponent of artificial cryptorchidism was the first to achieve effectiveness rates with it. In this method, during waking hours a man wears an under brief which holds the testes snug against the body but doesn’t tightly enclose the penis. The effectiveness shot up by adding a circle of soft fabric to keep the testes from moving out of the inguinal canal. This new method resulted in an average sperm count of 3 million/ml and average motility of 15 percent (Mieusset et al., 1991), as opposed to values with the old method of 12 million/ml and 22-30 percent motility (Mieusset et al., 1987).

POLYESTER SUSPENSORIES

This method is similar to artificial cryptorchidism underwear but is looser and made of polyester. Shafik (1992) showed that zero sperm could be obtained as a result of this method since polyester retains more heat.

ULTRASOUND

For ultrasound contraception, ultrasound waves (very short, inaudible sound waves) are used to heat the testes. Considered on technical and practical merits alone, ultrasound is one of the most promising forms of new male contraception. It is technically simple and extremely convenient in that ten minutes of ultrasound results in six months of sterility. With two treatments forty-eight hours apart, ten or more months of infertility will result (Fahim, 1980). To use this method, a man first sits in a special chair with his scrotum in a cup of water. In the bottom of the cup, shielded from the testes
and scrotum, is an ultrasound element, which heats the water somewhat and creates an ion exchange between the fluid in the seminiferous tubules and the rete testis which makes the environment in the testes inhospitable for sperm formation (Fahim et al., 1977).

TESTICULAR ANTI-SPERMATOGENIC AGENTS

The modest antifertility side effects of sulphapyridine has led to a search for analogous but more potent compounds which are inhibitors of dihydrofolate reductase. This revealed that the anti-malarial drug pyrimethamine at high doses reversibly abolished fertility in male rats. It is hoped that chemical modifications to the basic diaminopyrimidine structure would improve the testicular selectivity of these compounds (Maltin, 1994).

Two new chemicals (1-(2,4-dichlorobenzyl)-indazole-3-carboxyhydrazide and 1-(2,4-dichlorobenzyl)-indazol-3-acrylic acid exert their effects in the testis by perturbing the Sertoli-germ cell adherens junction causing germ cell loss from the seminiferous epithelium. Neither compound affected the hypothalamus-pituitary-testicular axis and both compounds were neither hepatotoxic nor nephrotoxic as shown in recently completed studies in the rat. Efficacy, reversibility and potential use of these two compounds as oral contraceptives for men was demonstrated (Yan et al., 2002).

POST-TESTICULAR CONTRACEPTION

The post-testicular or epididymal approach has the benefits of (i) almost immediate effectiveness, (ii) ready reversibility and (iii) avoidance of psychological or
endocrine impairment of libido. Within the epididymis, contraceptive effects would be mediated on the spermatozoa directly, via the epididymal epithelium on epididymal fluid composition or on epididymal peritubular muscle.

α-chlorohydrins and 6-chloro-6-deoxyglucose are both metabolized to 3-chlorolactaldehyde, the s-enantiomer of which has the same absolute stereochemistry as R-glyceraldehyde, the substrate for the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase. These compounds inhibit oxidative metabolism of glucose and other sugars in the spermatozoa resulting in loss of sperm motility. Unfortunately, the serious adverse effects of these compounds on the nervous system and bone marrow precluded further development (Ford and Waites, 1986).

The potential for successful interference with maturation of spermatozoa within the epididymis is suggested by the effect of c-ros tyrosine kinase knockout in mice (Sonnenberg-Riethmacher et al., 1996).

After oral administration of the alkylated imino sugar N-butyldeoxynojirimycin (NB-DNJ) to mice, epididymal spermatozoa displayed a spectrum of abnormal head shapes, and acrosomal antigens were mostly absent or displayed irregular patterns. Biochemically the capacity of imino sugars to impair spermatogenesis was associated with their potential to attenuate the biosynthesis of glucosylceramide-based sphingolipids (Van der Spoel et al., 2002).

**PLANTS AND PLANT PRODUCTS**

A marked growth in the world wide phytotherapeutic market has occurred over last 15 years. For the European and USA markets alone, this reached about $7 billion and
$5 billion per annum, respectively in 1999 and has thus attracted the interest of most large pharmaceutical companies (Calixto, 2000). Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Inspite of great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants (Farnsworth and Morris, 1976; De Smet, 1997; Cragg et al., 1997; Shu, 1998).

A multiglycoside extract of plant *Tripterygium wilfordii*, long used in Chinese traditional medicine for the treatment of psoriasis was shown to cause reduction in sperm motility and concentration in male patients (Qian, 1987). A series of diterpene epoxides from extracts of root bark of the plant were isolated. Several were shown to be orally active in rats in exceedingly low doses. One triptolide was found to induce complete infertility in male rats acting primarily on epididymal sperm with minimal effects of the testis (Lue et al., 1998).

Antifertility activity of *Cuminum cyminum* was reported by Al-Kharnis and Al-Said (1988) and Sharma et al. (2001).

Reddy et al. (1997) have shown decrease in spermatogenic elements of testis and epididymal sperm count due to benzene, chloroform and alcoholic extracts of flowers of *Hibiscus rosa sinensis*. Chinoy et al. (1995) have shown the contraceptive efficacy of *Carica papaya* seed extract in male mice (*Mus musculus*). *Carica papaya* seed extract which showed contraceptive efficacy in animal models, also showed sperm immobilization effects *in vitro*, in human spermatozoa showing potential as vaginal spermicidal contraceptive (Lohiya et al., 2000). Gupta et al. (2000) have reported
antifertility studies of the root extract of the *Barleria prionitis* Linn in male albino rats. Verma et al. (2002) have reported antispermatogenic/antiandrogenic effects of *Sarcostemma acidum* stem in male albino rats without affecting general body metabolism.

**HORMONAL METHODS**

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

**ANDROGENS**

In the first large scale efficacy trial of male hormonal contraceptions, it was demonstrated that weekly injections of 200 mg testosterone enanthate (TE) administered continuously over one year were as effective as female hormonal contraceptives, with a Pearl index of 0.8 (WHO, 1990). Administration of high dose testosterone (T) causes profound gonadotropin suppression and results in azoospermia in 50-70% of the subjects (Paulsen et al., 1982; Matsumoto, 1990).

Testosterone buciclate (TB) provided stable testosterone concentrations over prolonged periods in non-human primates (Weinbauer et al., 1986; Behre et al., 1995;
Rajalakshmi et al., 1995) and suppressed spermatogenesis in contraceptive studies (Behre et al., 1995). Testosterone buciclate (20 Aet-1) shows favourable pharmacokinetics and pharmacodynamics. This new long acting T ester is a promising new agent for male contraception (Behre and Nieschlag, 1992). In a study of male contraceptive potential of new long acting T ester TB in normal men, it could be shown that a single injection of 1200 mg TB leads to suppression of gonadotropins and to azoospermia in three of eight volunteers (Behre et al., 1995).

Multicentre clinical trials to determine the contraceptive efficacy of hormonal methods of male fertility regulation showed that administration of testosterone enanthate at weekly intervals resulted in azoospermia and severe oligozoospermia in most men, which was associated with acceptably low pregnancy rates in their partners (WHO, 1990; WHO, 1996).

Handelsman et al. (1992) reported sperm output to near-azoospermia between second to fourth post-implant months after insertion of six 200 mg testosterone implants under the abdominal wall skin in men.

A new injectable formulation of Testosterone undecanoate (TU) in tea seed oil provides more stable long-term release of T into the circulation (Chen et al., 1991; Li et al., 1994).

A dose-finding study of TU for spermatogenic suppression demonstrated that monthly injection of either 500 or 1000 mg TU to normal Chinese men can sufficiently and reversibly suppress spermatogenesis without serious adverse effects (Zhang et al., 1998).
O'Donnell et al. (2001) reported on impairment of spermatogonial development and spermiation after Testosterone-induced gonadotropin suppression in adult monkeys (Macaca fascicularis). MENT (7α Methyl-19-Nortestosterone) may have a wider therapeutic index than testosterone for human androgen replacement and male contraception (Cummings et al., 1998).

ESTROGENS

All estrogens are related to the parent structure estrone (18 carbons) and possess an aromatic A ring and certain oxygenated substituents at C-3 and C-17. A number of potent estrogens have been synthesized and are used orally or parenterally. Structural modification of estradiol molecule by insertion of an ethinyl group at C-17 results in formation of ethinyl estradiol, a very potent estrogen with high oral activity. Long acting, oil soluble estrogenic preparations used therapeutically are synthesized by esterification of estradiol at C-3 or C-17 with fatty acids. In general the higher the molecular weight of estradiol derivative, the more pronounced is the biological activity of the compound.

Estrogens are potent inhibitor of gonadotropin release in the male, inhibiting spermatogenesis and reducing testosterone secretion. They also tend to reduce libido. The inhibitory effect of estrogen on testicular steroidogenesis is well demonstrated (Dufau et al., 1978). Rao and Chinoy (1984) and Rao et al. (1993; 1994) showed its effects on male reproductive system using diethylstilbesterol (DES) and androgen combination. Estrogenic compounds have been used as a potent antispermaticogenic and antifertility agents in males (Rao and Chinoy, 1983). The potential contraceptive efficacy of a combination of testosterone and estradiol 17β or DES has been suggested by Ewing et al.
(1977) and Rao et al. (1993; 1994). The results suggested that the rats were 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). Estradiol benzoate (E₂B) which is formed by esterification of estradiol with benzoic acid showed androgen antagonistic and antifertility effects in rats (Chinoy and Rao, 1982; Rao and Chinoy, 1983; 1984). Eddy et al. (1996) based on their studies on ER knock-out; ERKO mice suggested that estrogen action is required for fertility in male mice and that the mutation of the ER in ERKO males leads to reduced mating frequency, low sperm numbers, and defective sperm function.

In the male, estrogen regulates one of the most important epithelial ion transporters and maintains epithelial morphological differentiation in efferent ductules of the male, independent of its regulation of Na(+) transport. Finally raising the possibility of targeting ER alpha in developing a contraceptive for the male (Zhou et al., 2001). Handelsman et al. (2000) have reported that estradiol enhances testosterone-induced suppression of human spermatogenesis.

**ANTI-ANDROGENS**

They are compounds which prevent the expression of biological activity of androgens at target sites. They inhibit or block the androgenic effect of testosterone at central and peripheral receptors. They 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 diminishes.
Combined administration of cyproterone acetate (CPA) plus low dose testosterone enanthate (TE) was proved to induce rapid and profound sperm suppression (Meriggiola, et al., 1996; 1998; 2002). Decrease in sperm density and in normal-shaped sperm were seen as an effect of CPA administered orally in doses of 10 or 20 mg/day for 26 weeks in healthy male volunteers (Koch et al., 1976).

PROGESTINS

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

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.

Levo-norgestrel in combination with testosterone oenanthate caused reduction in sperm count in males (Fogh et al., 1980). Sharma and Kanwar (1983) tested the effects of megastrol acetate (MA) and medroxy progesterone acetate (provera) in combination with methyl-testosterone (MT) and testosterone enanthate (TE) respectively. They reported that one milligram of progestin and 100 micrograms of androgen, when administered for 70 days, impaired the fertility in male rat without any apparent effect on mating behaviour. Danazol (15 mg/kg b.wt./day; oral) in combination with testosterone enanthate (5 mg/kg b.wt./15 days; s.c.) to male rabbits caused reversible inhibition of spermatogenesis (Lohiya and Sharma, 1984a). NET acetate also showed promising
results when combined with T gel application (Guerin and Rollet, 1988). Bimonthly injections (im) of medroxy progesterone acetate (MPA 5 mg/0.2 ml) and testosterone enanthate (TE, 2.5 mg/0.2 ml) to male rats for 30 and 60 days induced oligospermia (Rao and Roy, 1993). Bebb et al. (1996) concluded that combination hormonal therapy with T plus a progestogen might offer a reversible male contraceptive approach with a more rapid onset of action and more reliable induction of both azoospermia and severe oligospermia than T alone. Wu et al. (1999) concluded from their study that combined actions of oral desogestrel (DSG) with low doses of Testosterone enanthate (TE) were highly effective in suppressing pituitary-testicular functions in adult men. Anawalt et al. (1999) concluded that the combination of physiological exogenous T enanthate and levonorgestrel suppresses spermatogenesis more effectively than T enanthate alone and that the combination regimen of T enanthate plus lower dosage LNG suppresses sperm production comparably to T enanthate plus higher dosage LNG. Thus, it offers great promise as a safe and effective male contraceptive regimen. Anderson et al. (2002) reported that etonogestral implants with depot testosterone provide effective suppression of spermatogenesis with reduced metabolic effects and therefore a promising approach to the development of long acting yet reversible male contraception. Suppression of spermatogenesis to azoospermia in 13 of 14 volunteers was achieved with combination of TU and nor ethisterone (NET) (Kamischke et al., 2001). Gaw-Gonzalo et al. (2002) reported that Norplant II (Levonorgestrel) implants plus TE (100 mg/wk) were very efficient in suppressing spermatogenesis to a level acceptable for contraceptive efficacy.

Testosterone (four 200 mg implants every 4 or 6 months) and 300 mg depot medroxy progesterone acetate, im, every 3 months caused contraceptive effect. No
pregnancies occurred in 426 person-months (35.5 person-years; 95% confidence limits for contraceptive failure rate, 0-8%/annum) (Turner et al., 2003).

**GnRH ANALOGS**

Sperm density decreased significantly in normal male volunteers as a result of treatment with a LHRH analog, 100 to 500 micrograms daily for 20 weeks and testosterone enanthate 100 mg, every second week (Rabin et al., 1984). Since the isolation and chemical characterization of GnRH in 1971, more than 3000 agonistic or antagonistic analogs of GnRH have been synthesized for possible medical application (Matsuo et al., 1971; Struthers et al., 1990; Karten and Rivier, 1986).

**AGONIST**

83% decline in mean sperm count was reported (Bhasin et al., 1985) as a result of treatment of 200 micrograms of GnRH agonist D-(Nal 2) 6 GnRH (GnRH-A), sc, for 16 weeks and 200 mg Testosterone enanthate every 2 weeks. The antigonadal effects of GnRH agonists in humans and non-human primates can be attributed entirely to the inhibition of pituitary gonadotropin secretion by down-regulation of pituitary GnRH receptors and secretion of gonadotropins whose molecular structure has been altered (Bhasin and Swerdloff, 1986). Clinical studies applying GnRH agonists for male contraception failed to achieve azoospermia in a high proportion of participating volunteers (Weinbauer et al., 1990).
ANTAGONISTS

GnRH antagonists are synthetic analogs of GnRH that compete with endogenous GnRH for pituitary binding sites, thereby causing immediate suppression of gonadotropin secretion (Karten and Rivier, 1986).

Rivier et al. (1980) reported that administration of a potent antagonist of gonadotropin releasing hormone (GnRH) antagonist [Ac-dehydro-Pro1, p-e1-D-Phe 2, D-Trp 3,6] – N – alpha MeLeu7-GnRH to adult male rats for two weeks resulted in decreased testosterone production and sexual organ weights and in disrupted spermatogenesis and thus regulation of male fertility.

Complete sustained azoospermia could be achieved in man, without loss of libido, by treatment of GnRH antagonist Nal-Glu given daily (10 mg, sc) for 20 weeks and testosterone enanthate (25 mg, sc) every week (Pavlou et al., 1991).

Combined administration of potent Nal-Glu GnRH antagonist ([Ac-D2-Nal1, D4-C1-Phe2, D3-Pal3, Arg5, D4-p-methoxybenzoyl-2-amino butyric acid 6, D-Ala10] GnRH) (7.5 mg, sc) and TE im, every two weeks could predictably and reversibly induce azoospermia in most men and has potential as a male contraceptive regimen (Tom et al., 1992).

New GnRH antagonist cetorelix (SB-75) effectively decreases serum LH and testosterone concentrations in a dose and time-dependent manner and therefore, has potential for treatment of sex hormone – dependent diseases and male contraception (Behre et al., 1992).
Sperm motility in normal men showed significant decrease due to treatment with 10 mg GnRH antagonist every day and 25 mg T enanthate once a week. Azoospermia was reached within 6 to 12 weeks of GnRH administration and was sustained during treatment period (Bastias et al., 1993).

Hormone antagonist, Nal-Glu, at a dose of 200 µg/kg per day sc in combination with Testosterone enanthate (TE), 50 mg im weekly suppressed gonadotropins in normal men (Bagatell et al., 1995).

GnRH antagonists are expensive to synthesize and difficult to deliver, it is desirable to rapidly suppress sperm counts to low levels with GnRH antagonist plus T and maintain azoospermia or severe oligozoospermia with T alone. This concept can be further explored in the development of effective, safe and affordable hormonal male contraceptives (Swerdloff et al., 1998).

**IMMUNOCONTRACEPTION**

Luteinizing-hormone-releasing hormone (LHRH) was coupled to diphtheria toxoid (DT) and male adult Sprague-Dawley rats were immunized with anti-LHRH-DT (20 µg/injection/rat) at four week intervals. The weights of reproductive organs demonstrated significant reductions compared to those of control group. If the vaccination is given together with a suitable dose of long acting androgen, contained in an adequate delivery system, the regimen may be used for the regulation of male fertility (Rovan et al., 1992).

Birth control vaccines, both would increase the choice of methods open to women and men and additionally would offer specific advantages, including the development of
vaccines that are unlikely to disrupt the menstrual cycle or cause the side effects associated with oral contraceptive (OC) use.

For a birth control vaccine to be acceptable for use in human beings, it must meet several criteria: the antigen must be unique to the reproductive target, the antigen must have a fertility related function that can be blocked by antibody or is susceptible to cell-mediated immunity, and alternatively the function should be located on a cell that can be lysed by complement, an acceptable level of effectiveness should be achieved by no more than 1 or 2 injections for primary immunization, with booster injections at intervals of no less than 6-12 months and the vaccine must undergo sufficient testing in animals to ensure its safety for long term use.

The WHO special program on Research Development and Research Training in Human Reproduction, through its Task Force on vaccines for Fertility Regulation has been supporting research on a synthetic vaccine directed against the last 37 amino acids of the C-terminal end of the beta hCG molecule.

A second approach being followed by the National Institute of Immunology, New Delhi, and the Population Council, New York, involves developing a vaccine against the entire beta chain of hCG molecule. A prototype vaccine using the beta subunit of ovine luteinizing hormone emulsified with Freud's complete adjuvant has been studied extensively in female primates by the Population Council, but as yet there is no evidence of acute or chronic health hazards in this heterologous immunization model (Spieler, 1987).

Another possibility is disrupting the synthesis or delivery of proteins such as fertilin that are important for the function of sperm membrane, thus leading to
incompetent spermatozoa. Interfering with the final maturation of the spermatozoa has the attraction that it would result in sperm that were incompetent to fertilize an egg without running the risk of producing genetically mutated germ cells. Due to concerns and doubt about long term consequences of immunization, there is little commercial enthusiasm for further development of this approach in spite of the scientific potential (Herr, 1996).

**GnRH VACCINE**

Passive as well as active immunization against GnRH has been shown to suppress release of gonadotrophins thereby resulting in the regression of Leydig cells and suppression of testosterone production and spermatogenesis in rodents and non-human primates (Pal and Talwar, 1995).

Active immunization of adult male bonnet monkeys (*Macaca radiata*) with purified ovine FSH, adsorbed on an adjuvant alhydrogel produced high titre antibodies capable of neutralizing hFSH (Moudgal et al., 1992). Immunization resulted in reduction in sperm counts to acute oligozoospermia to azoospermia without causing any significant change in the serum testosterone levels. Mating studies with proven fertile female monkeys revealed that none of the male were able to impregnate females, thereby demonstrating that FSH immunization had rendered the males infertile. Immunization caused reduction in sperm counts (30-74%) in some volunteers. An extended phase 1 clinical trial evaluating the safety and efficacy of oFSH derived vaccine is warranted.

Immunization against LH might be expected to be similarly efficacious, although by reducing testosterone production, this approach requires the concomitant
administration of testosterone to prevent hypogonadal symptoms, as with the hormonal approach. Marked inhibition of spermatogenesis was achieved in non human primates by active immunization against ovine LH (Suresh et al., 1995).

A number of spermatozoal antigens have been investigated as immunological targets in animal models, some with promising results (Naz, 1999). Immunization against one such antigen, PH-20, induced reversible infertility in all male guinea pigs treated, with infertility lasting longer than 1 yr in some cases (Primakoff et al., 1997). Some spermatogenic antigens are proteins acquired by the spermatozoa during their passage through the epididymis and may be required for fertilization (Cohen et al., 2000). The identification of human proteins with similar roles (Cohen et al., 2001) raises the possibility for clinical studies in both men and women. The identification of these specific epididymal proteins and elucidation of their functions also raises the potential for their targeting by pharmacological agents. One problem specific to epididymal antigens is that the blood testis barrier may prevent adequate titers from reaching the epididymal lumen.

MALE REPRODUCTIVE SYSTEM

The male reproductive system consists of testis, epididymis, vas deferens and other accessory sex organs like the seminal vesicles, prostate glands, coagulating gland, cowper’s glands, preputial glands and penis (Sarkar, 1996).
The testes in all mammals are paired encapsulated ovoid organs consisting of seminiferous tubules separated by interstitial tissue. The degree of lobulation of the testis varies between species, and within these lobules lie the seminiferous tubules, within which spermatogenesis occurs.

The mammalian vertebrate testis have been evolved to perform dual functions of being both exocrine and endocrine. Its primary function is to produce sperms, and other is to secrete the male sex hormones, the androgens, which regulate spermatogenesis, development and differentiation of accessory reproductive organs and synchronize their functional physiology. The vascularized interstitial tissue containing the cells of Leydig produce androgens. The epithelial lining of the seminiferous tubule is called seminiferous epithelium which contains two types of cells. An interdependent mass of the proliferating cells, the germ cells produce sperm. The second type of the cells are the non-proliferating group of irregularly shaped sessile Sertoli cells. They provide architectural support to the tubules and also a micro-environment for the germ cells undergoing the process of sperm formation.
The interphase germ cells of the immature testis are re-activated at puberty to enter rounds of mitosis. Henceforth they are known as spermatogonia. This re-entry of the resting spermatogonia into mitosis marks the beginning of the process of spermatogenesis. Each of the activated spermatogonia is in the basal compartment of the
tubule and undergoes a limited number of mitotic divisions at about 42-hour intervals, thus producing a 'clone' of daughter cells. In the rat there are six divisions leading to a maximum clone size of 64 cells, but since appreciable numbers of cells die during mitosis, the full sized clone is not actually achieved. Spermatogonia in the rat are subclassified as being 'type A' during the first three mitoses, 'type intermediate' after the fourth mitosis and 'type B' after the subsequent fifth division. All the spermatogonia type B of daughter clone will synthesize another round of DNA and divide. Their progeny are resting primary spermatocytes. These cells enter into meiotic prophase. At some point during the mitotic divisions of the type-A spermatogonia, one of the daughter cells of the clone does not divide and differentiate to generate resting spermatocytes. These cells serve as a stem cell that at a later time, will enter into the same sequence of six divisions afresh. During the proliferative phase of spermatogenesis the mitotically dividing cells are present in the basal intratubular compartment of the testis. Within this compartment the clone of resting primary spermatocytes, derived from each stem cell, duplicate their DNA content and then push their way into the adluminal intratubular compartment by transiently disrupting the zonular tight junctions between adjacent sertoli cells. Within the new and distinctive micro-environment of the adluminal compartment, they then enter the first and prolonged meiotic prophase. The first meiotic division ends with the separation of homologus chromosomes to opposite ends of the cell on the meiotic spindle, after which cytokinesis yields, from each primary spermatocyte, two daughter secondary spermatocytes, each containing a single set of chromosomes. Each chromosome is comprised of two chromatids joined at the centromere. The chromatids then rapidly separate at their centromere and move to opposite ends of the second meiotic spindle, and
the short-lived secondary spermatocytes divide to yield haploid early spermatids. The spermatid DNA becomes highly condensed and packed with nuclear basic proteins into tight, inactive units of chromatin. Generation of tail for forward propulsion, the midpiece containing the mitochondria and acrosome and the residual body. This process of spermiogenesis is complex and not all spermatids complete it successfully. As meiosis and spermiogenesis proceed, the spermatogenic cells are moved slowly towards the lumen of the tubule until, with the completion of spermatid elongation, the sertoli cell cytoplasm around the cells suddenly retracts. Any junctional contacts are broken and the elongated spermatids are released into the lumen of the seminiferous tubule bathed in the distinctive tubular fluid. These newly formed, immature spermatozoa will then be carried away by the fluid through the excurrent ducts of the testis at the start of their journey towards the egg (Johnson and Everitt, 1980).

**EPIDIDYMIS**

The ductus epididymis is a single highly convoluted duct, closely applied to the surface of the testis extending from the anterior to the posterior pole of that organ and held more or less firmly to the tunica albugenia by connective tissue. The segment into which the ductuli efferentes empty is usually referred to as the initial segment and the remainder of the epididymis is loosely defined into three parts termed the caput, corpus and cauda epididymidis. The initial and middle segments are primarily concerned with sperm maturation, whereas the terminal segment coincides with the region where mature sperm are stored prior to ejaculation or voidance into the urine. The epididymal epithelium is complex in, it contains a variety of cell types, each cell type varying as a
proportion of the total population at different points along the duct. The predominant cell type is the principal cell which bears apical stereocilia. Other cell types include apical cells, basal cell, clear cells and halo cells (intraepithelial lymphocytes). The first part of the epididymis or initial segment is characterized by a high epithelium with long straight stereocilia which almost obliterate the lumen which is sparsely populated with spermatozoa. The middle segment has a wider lumen and the stereocilia are usually bent and sometimes branched, while supranuclear vacuoles are prominent in the epithelium. The terminal segment has a lower epithelium, stereocilia are shorter and less dense and the lumen of the tubule is wider and densely packed with sperm. The epididymal tubule is surrounded by connective tissue which contains fibroblasts, collagen, elastic filres, blood vessels, lymphatic vessels, nerve fibres, macrophages, wandering leucocytes, and concentric layers of smooth muscle fibres. Within a species the thickness of the smooth muscle layer surrounding the tubules increases from the initial segment to the terminal segment.

**VAS DEFERENS**

The ductus (vas) deferens is a continuation of the epididymal duct beginning at the point where the ductus epididymides straightens and reverses direction towards the inguinal canal. The duct is approximately 6 cm in the rat, and is suspended in mesentry that is continuous with that over the epididymis. In the rat, the ductus deferens can be subdivided into three sections. The proximal vas deferens, located primarily in the scrotum, is flattened due to an asymmetric distribution of longitudinal muscle layers, but contains a tubule which is circular in cross section. The distal vas deferens, in the
inguinal region is circular in cross section due to the presence of thick longitudinal layers but the epithelium of the duct becomes crenelated with two to six infoldings, and the structural features of the epithelial cells differ from those in the proximal vas. The terminal region of the vas deferens lies in the abdominal pelvis and terminates at the point where it is joined by the duct of the seminal vesicles to form the short vesicles to form the short ejaculatory duct. It is characterized by replacement of columnar principal cells, in some areas, by pockets of smaller cells which can be seen actively to phagocytose spermatozoa. The epithelium of the human vas deferens is crenelated in more distal regions as in the rat and this produces stellate shape in cross section. Four different cell types are recognized in the epithelium, namely principal cells, pencil cells, mitochondria-rich cells, and basal cells. The muscle coat is composed of three layers, an inner longitudinal layer, a middle oblique or circular layer, and an outer longitudinal layer.

**PROSTATE**

The prostate, apparently so named due to its location anterior to the bladder and seminal vesicles, is present in essentially all mammalian species, but has a widely varying morphology. The prostate is a compound tubuloalveolar gland. The rat prostate is also a popular experimental system, particularly for studies of androgenic control of male accessory glands. The rat prostate is a complex structure with several distinct anatomical lobes. Its classification encompasses the ventral prostate, which is a bilobed structure situated ventral to the urethra, and the dorsolateral prostate, which is comprised of a clearly separate medial portion and two lateral lobes located over the dorsolateral aspect
of the urethra. The ventral, lateral, and dorsal lobes of the prostate are each drained into the urethra by multiple ducts.

**SEMINAL VESICLES**

The seminal vesicles are paired, bag shaped glands in man, stallion, rat and guinea pig, although the internal surface may be thrown into an intricate system of folds to form irregular diverticula. The epithelium is generally pseudostratified, consisting of a row of round basal cells and a row of larger low columnar cells. The remainder of the gland is completed by loose connective tissue, a layer of smooth muscle, and an external sheet of connective tissue.

**SPERM STRUCTURE**

Mammalian spermatozoa are small and motile and show a general uniformity in their internal and external structure. The sperm consists of two principal parts, head and the tail. The tail consists of four components such as the neck, mid-piece, principal piece and end piece (Phillips, 1975). The main part of the head is occupied by the nucleus which largely consists of closely packed chromatin material. The narrow region which connects the sperm head with the middle piece is known as the neck. The middle piece contains the primary chemical energy exchange mechanism, mitochondria, in the form of a sheath around the mid piece. These organelles are arranged in a lightly coiled spiral, surrounding the contractile fibrils which provide locomotion and originate in the neck of the spermatozoa and pass through the tail. The spermatozoon is a haploid cell which can be differentiated into male (Y) and female (X) bearing gametes. The acrosomal region is
covered by acrosomal membrane and is covered by plasma membrane. Acrosome is formed by the golgi apparatus during spermatogenesis. This region is particularly rich in hydrolytic enzymes. 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 propogating localized contractions along their length (Knobil and Neill, 1988).

RATIONALITY OF PRESENT WORK

PART-I

HORMONAL COMBINATION

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

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

Hormonal methods are the most advanced of contraceptive methods for men. Their development was much to the involvement of Asian centers and investigators. The suppression of sperm production by hormonal means is achieved by the suppression of the secretion of both LH and FSH or of FSH alone and the maintenance of androgen levels in the physiological range. Various drugs either alone or in combination have been tried (Waites, 1993; Cummings and Bremner, 1994). Two multicentered studies in which
men suppressed to azoospermia or severe oligozoospermia were conducted by WHO to establish the contraceptive efficacy rates (WHO, 1991; WHO, 1998).

All hormonal regimens capable of suppressing sperm production to the same degree as in WHO studies (WHO, 1991; WHO, 1996) should receive high levels of sustained and reversible contraceptive efficacy. Encouraging results of these studies have stimulated interest in the long acting drugs. Novel formulations of testosterone esters may be administered alone or in combination with progestagens such as DMPA.

MEDROXY PROGESTERONE ACETATE

Depot medroxy progesterone acetate (DMPA, Depo Provera; 17-alpha-hydroxy-6 alpha methyl progesterone acetate) is a progestagen and a derivative of progesterone. It is one of the most widely studied hormonal contraceptive 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 potent progestational steroid which possesses antiandrogenic, syndrogenic and glucocorticoid activities when tested in vivo (Bullock et al., 1978; Lin et al., 1978; Brown et al., 1979).

CHEMISTRY

PHARMACODYNAMICS

Medroxy progesterone acetate is a 17-acetoxy, 6 Methylpreg-4-ene-3,20 dione. It belongs to class of C-21 steroids. It has a very close structural similarity to natural progesterone. Like progesterone, medroxy progestone acetate is thermogenic. It is a progestational agent devoid of estrogenic activity. It is prepared as a sterile aqueous microcrystalline suspension for intramuscular injections. Each ml contains Medroxy progesterone acetate 150 mg - polyethylene glycol 3350-polysorbate 80-sodium chloride-Methylparaben-propylparaben water for injection. The unusual stereochemistry of crystal structure seems to be important for its slow release (Duan et al., 1978) into the blood. As it is not metabolized as rapidly as its parent compound, progesterone, MPA can be given in smaller amounts than progesterone with an equivalent progestational activity. DMPA, the long acting injectable formulation of MPA, consists of a crystalline suspension of this progestational hormone.
PHARMACOKINETICS

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

MODE OF ACTION

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

In normal men, serum concentration of LH, FSH and testosterone had registered a decrease following treatment with DMPA (Rivarola et al., 1968; Faundes et al., 1981). Thus, the efficacy of DMPA might be due to its ability to suppress the circulating testosterone, for which three mechanisms had been proposed (Barbieri and Ryan, 1980) : (1) by increasing the metabolic clearance rate of testosterone; (2) by decreasing the circulating gonadotropins and (3) by directly interfering with Leydig cell steroidogenesis. It was demonstrated that DMPA induced ring reductase activity in rat liver (Altman et al.,
Hence it was postulated that DMPA increased the metabolic clearance of testosterone. A similar increase was reported in normal men and women (Gordan et al., 1970) but other experiments in men treated with DMPA failed to show an increase (Norten 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 gonadotropin concentration by about 25-50% was reported in case of boys and men treated with DMPA (Rifkind et al., 1964; Meyer et al., 1977).

The direct effect of DMPA on Leydig cell steroidogenesis could be attributed to the fact that a large reduction in plasma testosterone without plasma LH or FSH being altered, was noticed in a boy with hypothalamic hematoma secreting LHRH, after the administration DMPA (Judge et al., 1977) and the direct effect of DMPA on Leydig cell steroidogenesis could also be due to suppression of 17β hydroxy steroid dehydrogenase activity (Satyaswaroop and Gurpide, 1978; Rao et al., 1995; Roy and Rao, 1995) similar to in vitro studies of Barbieri and Ryan (1980).

**TOXICOLOGY**

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

The toxicological review panel saw no reason to alter its opinion that MPA was safe for use in human beings (WHO, 1982). A large number of clinical trials including multicenter 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

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

As DMPA does not increase liver globulin production it is not associated with an alteration in blood clotting factors on angiotensinogen levels. DMPA has not been associated with increased incidence of hypertension or thromboembolism (Wilson et al., 1984). A WHO study reported that mean blood pressure measurements were unchanged in DMPA injection users of two years (WHO, 1982).
The oral glucose tolerance tests were performed on long term DMPA users and matched controls not using hormonal contraceptives (Lieu et al., 1985; Virutamasen et al., 1986). The mean glucose levels were slightly higher among DMPA users. Mean insulin levels were also higher. A slight deterioration in glucose tolerance among DMPA users is probably not clinically significant and returns to normal after use of DMPA is discontinued.

Handeleman et al. (1996) reported no significant effects of DMPA on cholesterol fractions (total, LDL, HDL) or triglycerides. Turner et al. (2003) also reported similarly that lipids (total, low density lipoprotein, cholesterol and triglycerides) did not get affected significantly by a depot progestin treatment in their study.

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

To determine the effect of DMPA on the hypothalamo-pituitary axis. Goldzieher et al. (1970) measured FSH and LH levels in single blood samples from women who received injections of DMPA every three months for as long as two years. In this cross sectional study, both FSH and LH levels remained in the mean range of those in the
control. Thus long term use of DMPA does not cause complete suppression of hypothalamo-pituitary axis.

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

**TESTOSTERONE ENANTHATE (TE) (C\textsubscript{26}H\textsubscript{40}O\textsubscript{3})**

Free unesterified testosterone as physiologically secreted by the testes would appear to be the first choice when considering substitution therapy. When ingested orally in the free form testosterone is absorbed well from the gut, but is effectively metabolized and inactivated in the liver before it reaches the target organs ("first pass-effect"). Several attempts have been made to modify the testosterone molecule by chemical means in order to render it orally effective, i.e. to delay metabolism in the liver eg: 17α-methyltestosterone, fluoxymesterone, mesterolone, testosterone undecanoate, testosterone
SINGLE DOSE PHARMACOKINETICS

Single dose pharmacokinetics of testosterone enanthate were studied in seven patients with primary hypogonadism, 3 castrates and 4 patients with klinefelter's syndrome, aged 20-58 years (Nieschlag et al., 1976). The usual androgen substitution therapy in these patients was discontinued at least 6 weeks before the investigation. 250 mg of testosterone enanthate was injected at 18:00 h on the control day. Blood samples were obtained at 08:00 h on the following test days. Maximal testosterone levels in the supraphysiological range were seen shortly after injection (39.4 nmol/l, t\text{max} = 10 h). Testosterone levels below the normal range were observed following day 12 after injection. The calculated values were 9911 n mol/l for AVC, 8.5 d for MRT and 4.5 d for terminal half-life.

MULTIPLE DOSE PHARMACOKINETICS

Based on the pharmacokinetic parameters of single dose pharmacokinetics multiple dose pharmacokinetic simulations for equal doses of 250 mg testosterone enanthate and injection intervals of 1 to 4 weeks were performed. With weekly injection intervals, supraphysiological maximal testosterone serum concentrations up to 78 n mol/l shortly after injection and supraphysiological minimal testosterone serum concentrations upto 40 n mol/l just before the next injection are observed at steady state. Injecting 250 mg of testosterone enanthate every two weeks results in maximal supraphysiological
testosterone serum concentrations up to 51 n mol/l shortly after injection and testosterone serum levels at the lower range for normal testosterone serum concentration (12 n mol/l) shortly before the next injection. If the injection interval is extended to 3 weeks, 14 days after injection testosterone serum concentrations below the normal range are observed. With injection intervals of 4 weeks, testosterone serum concentrations at week 3 and 4 are in the subnormal range and an effective androgen substitution is not guaranteed.

The calculated testosterone serum concentrations at steady state obtained by computer simulation correspond well to the results of published studies describing multiple dose testosterone enanthate pharmacokinetics. In a clinical trial for male contraception 20 healthy men were injected 200 mg/wk of testosterone enanthate for 12 weeks (Cunningham et al., 1978). Minimal serum concentrations of testosterone at steady state, i.e. the testosterone serum concentration just before the next injection, were measured at 31.2 n mol/l to 39.5 n mol/l after injection of 200 mg testosterone enanthate. These values fit well with the computer-calculated minimal testosterone serum concentrations of 40 n mol/l after multiple injections of testosterone enanthate in a dosage of 250 mg/wk.

Snyder and Lawrence (1980) administered 100 mg/wk (n=12), 200 mg/2 wks (n=10), 300 mg/3 wks (n=9) and 400 mg/4 wks (n =6) testosterone enanthate to hypogonadal patients during a study period of 3 months. Blood was drawn during the last injection period, when steady state had been reached, every day (100 mg/wk) upto every 4th day (400 mg/4 wks). Similar to the computer simulation described above for 250 mg testosterone enanthate and injection intervals of 1 to 4 weeks, initial supraphysiological testosterone serum levels were seen shortly after injection. In the 100 mg/wk treatment
group, where daily blood sampling was performed, mean peak serum concentrations were seen 24 h after injection. Comparable to the results of the computer simulation, after injection of 200 mg/2 wks testosterone enanthate, following initial supraphysiological testosterone serum levels values fell to progressively lower values before the next injection, eventually reaching the lower normal limit. Similar results were described after injection of 300 mg/3 wks or 400 mg/4 wks testosterone enanthate. The authors conclude that the testosterone enanthate doses of 200 mg have to be injected every two weeks or of 300 mg every 3 weeks to guarantee an effective substitution therapy.

Demisch and Nickelsen (1983) deduce from their studies with testosterone enanthate for testosterone replacement therapy that if a dose of 250 mg testosterone enanthate once every three weeks is used, the concentration of both, total and ‘free’ testosterone are sufficiently high in the first and second week. In the third week however, total testosterone moves to the lower limit of the male range and erectile disturbances were reported by the patients (Nieschlag and Behre, 1990).

**PART-II**

India has a vast and nearly inexhaustible resource of drugs of plant origin. The systematic investigation of drugs in indigenous medicine in India on modern scientific lines was started more than thirty years ago. It is well known that medicinal plants are sources of many biologically active ingredients which cannot be ignored by advanced medical practice (Satyavati et al., 1987; Desta, 1994).
**ABRUS PRECATORIUS**

The plant belongs to the family ‘Fabaceae’. Its synonyms are *Abrus minor*. It is known in different languages as:

- English: Indian liquorice
- Sanskrit: Krishna gunja
- Gujarati: Chanothi
- Tamil: Gundu-mani
- Telugu: Guriginja
- Kannada: Gulganganji

*Abrus precatorius* is a slender, perennial climber that twines around trees, shrubs and hedges. It has no special organs of attachment. Leaves are glabrous with long internodes. It has a slender branch and a cylindrical wrinkled stem with a smooth textured brown bark. Leaves alternate, compound paripinnate with stipules. Each leaf has a midrib from 5 to 10 cm long. It bears from 20 to 24 or more leaflets, each of which is about 1.2 to 1.8 cm long, oblong and obtuse. It is blunt at both ends, glabrous on top and slightly hairy below. Flowers are small and pale violet in colour with a short stalk, arranged in clusters. The ovary has a marginal placentation.

The fruit, which is a pod, is flat, oblong and truncate-shaped with a sharp deflexed beak, is about 3 to 4.5 cm long, 1.2 cm wide, and silky-textured. The pod curls back when opened to reveal pendulous seeds. Each fruit contains from 3 to 5 oval shaped
seeds, about 0.6 cm. They are usually bright scarlet in colour with a smooth, glossy texture, and a black patch on top.

It is a wild plant that grows best in fairly dry regions at low elevations. It grows in tropical climates such as India, Sri Lanka, Thailand, the Phillippine Islands, South China, tropical Africa and the West Indies.

Roots and leaves contain glycyrrhizin the principal constituent of liquorice and their decoction is given for coughs and colds. The leaves are ground with lime and applied on acne sores, boils and abscesses. The seeds contain abrin, a poisonous principle similar to ricin from castor seeds.

Abrin, which consists of abrus agglutinin, and toxic lectins abrins [a] to [d] are the five toxic glycoproteins found in the seeds. Abrus agglutinin is a tetramer with a molecular weight of 134,900. It is non-toxic to animal cell and a potent haemagglutinator. Abrins a through d (molecular weight : 63,000 – 67,000) are composed of two disulphide-linked polypeptide chains. The larger sub-unit, which is the neutral B-chain has a molecular weight of approximately 35,000 (Windholz, 1983).

Pure abrin is a yellowish-white amorphous powder. The toxic portion is heat-stable to incubation at 60°C for 30 minutes. At 80°C most of the toxicity is lost in 30 minutes (Budavari, 1989). Abrin is soluble in sodium chloride solutions, usually with turbidity. The seeds also contain an amino acid known as abrine (N-methyl-L-tryptophan), glycyrrhizin and a lipolytic enzyme.

Rao (1987) has reported antifertility effects of alcoholic seed extract of *Abrus precatorius* in male rats. Sinha (1990) has reported post-testicular antifertility effects of *Abrus precatorius* seeds. Ratnasooriya et al. (1991) have reported sperm antimotility
properties of a seed extract of *Abrus precatorius*. They examined the inhibitory effects of a methanol extract of *Abrus precatorius* seeds on the motility of washed human spermatozoa.

From the above literature, it is clear that long-term effects of hormonal combination needs to be done, to find out side effects if any. Aqueous herbal product of Abrus also requires systematic study to investigate its anti-fertility potential in the male. Hence, this study is proposed to be undertaken in the male adult rodent model.