REVIEW OF LITERATURE
In the past, a large number of compounds have been screened for their potential as effective, safe and reversible male contraceptives. These include both steroidal (e.g. estrogens, androgens, progestins, and their synthetic derivatives) and non-steroidal (e.g. \(\alpha\)-chlorohydrin, WIN 18446, chlorinated sugars, insecticides, LHRH analogues, plant derivatives etc.) compounds. Antifertility effects have been found in these compounds to varying degrees, with and without adverse side effects.

**Steroidal compounds**

Testosterone and its esters have been worked upon by a number of workers as prospective male contraceptives.

Intramuscular injections of testosterone propionate given daily (25 mg/day) for 60 days to 7 men resulted in azoospermia (Reddy and Rao, 1972). There was no loss of libido or potency and sperm counts in all subjects returned to pretreatment levels by 150 days after discontinuation of treatment.

Abdi and Hasan (1973) reported no histological changes after 15 days of treatment with intramuscular injections (5 mg/day) of testosterone propionate. But
after 30 days about 60% of the tubules revealed disrupted spermatogenesis at spermatocyte stage. After 45 days, the above effect was more pronounced and widespread. However, after 60 days, signs of recovery were noticed, probably due to direct action of testosterone on germinal epithelium.

Mauss et al. (1975) administered 250 mg of testosterone enanthate (TE) weekly to seven men for 21 weeks. They noted that sperm counts were suppressed although consistent azoospermia was not reached. Serum testosterone almost doubled and FSH and LH levels were suppressed.

In two studies, weekly injections of 200 mg of TE were found to reduce sperm counts to less than 100,000/ml without elevating serum testosterone level (McClure et al., 1977; Steinberger and Smith, 1977).

Cunningham et al. (1977) who administered 200 mg TE/week for 12 weeks parenterally to 17 men reported severe azoospermia in most of the subjects. Mean serum testosterone levels were also elevated by 50% in the treated subjects vis-a-vis the control. Serum FSH and LH were reportedly suppressed. No side effects except slightly elevated SGOT were reported by these workers.
Paulsen et al. (1978) treated a group of 42 men with 200 mg of TE per week for 6 months. Sperm counts fell considerably and some subjects showed complete azoospermia too.

Nieschlag et al. (1978) investigated the effects of orally active testosterone undecanoate on spermatogenesis in seven men. A dose of 80 mg thrice a day for 10-12 weeks caused azoospermia in one man and reduced sperm count by 40% in four others. Serum testosterone, LH and FSH levels were reduced to varying degrees. Libido and potency was not affected and neither was hepatic function.

Swerdloff et al. (1978) administered 200 mg of TE weekly for 16 weeks to 17 subjects. Azoospermia was induced in 10 subjects, one had sperm count below 0.3 million/ml, one between 0.3 and 5 million/ml, whereas in the remaining five subjects sperm count did not fall below 5 million/ml. No significant side effects were noted following TE administration.

Steinberger et al. (1978) treated 18 subjects with 200 mg of TE twice weekly for 2 weeks and thereafter weekly for 2 weeks. Subsequently, 200 mg of TE was administered at intervals of 10-21 days. Sperm counts in the treated subjects were suppressed to oligospermic/azoospermic levels.
Plasma testosterone was 2-3 times the control value for the first 2 weeks but returned to normal when frequency of TE injections was reduced. Serum LH and FSH levels were reduced significantly during the first two weeks only, though these remained low as compared to the control all along.

Flickinger (1978) tried to find out the testicular cells affected during suppressed fertility due to androgen treatment. Rats were administered 120 μg TE/100 g body weight thrice a week. Infertility occurred after 11 weeks of treatment. After eight weeks, tubules contained few or no spermatids.

Weekly intramuscular injections of 200 mg TE caused azoospermia in 50% of the subjects and severe oligospermia in another 39% according to Paulsen et al. (1980). According to them, azoospermia took 7-20 weeks to develop.

Bansal and Davies (1986) have described that in mice, 80 or 160 μg of TE administered subcutaneously 3 times per week for 8-12 weeks reduced testis weight and increased seminal vesicle weight. Radioimmunoassay further indicated that such treatment increased serum testosterone concentrations. Treatment with TE, according to these workers,
decreased the number of step 16 and 7 spermatids, pachytene spermatocytes and type A spermatogonia and particularly reduced the proportion of step 7 spermatids which matured to form step 16 spermatids.

For the control of fertility, oral administration is preferred over parenteral administration since injections cause discomfort and are not convenient. But out of the currently available steroidal oral preparations, methyltestosterone and fluoxymesterone are not considered suitable for the purpose as these have proved to be hepatotoxic. Two other orally active androgens - mesterolone and testosterone undecanoate do not appear to be hepatotoxic (Hirschhauser et al., 1975) but do not seem fit for fertility control by themselves as these adversely effect libido, for the restoration of which these androgens are used in combination with progestins (Davies, 1983).

Androgens, though potent as male sterilants, are not suitable to be used alone as antifertility agents in men because pituitary inhibition and hence infertility will occur only if sufficient hormone is administered exogenously, to raise the plasma androgen concentration above normal level. But such a treatment if resorted to, might result in prostatic hypertrophy and other undesirable metabolic changes (Davies, 1983).
Antiandrogens and combinations

Antiandrogens are substances which interfere with the action of androgens (endogenous or exogenous), on target organs. The progestational compounds, including most of the antiovulatory steroids, appear to have less consistent effects in males. Antispermatic effects and depression of androgen-dependent parameters suggest gonadotrophin suppressing effects of these compounds in several species (Setty and Kar, 1967, 1968, 1969). However, direct hormonal effects too cannot be overlooked.

Progestagens are known to be capable of inhibiting the production/release of gonadotrophins. The decrease in the weight of accessory reproductive organs accompanied by a decrease in the fructose concentration is probably due to the antiandrogenicity of steroids at the level of pituitary-gonadal axis (Heller et al., 1958).

Heller et al. (1959) demonstrated that some progestagens viz. 17-alpha-ethyl-19-nortestosterone (Nilevar), 17-alpha-ethyl-19-nortestosterone (Norlutin) and 17-alpha-ethyl-17-hydroxyestrone-3-one (Enovid) given orally caused azoospermia in men within 4-10 weeks. They also observed a marked decrease in the diameter of
the seminiferous tubules, increased thickness of peritubular membrane and disappearance of Leydig cells. But as in case of estrogens, in almost all cases, gynaecomastia occurred along with loss of libido and potency.

Noradrenaline, taken daily for varying periods of time produced azoospermia in men. Direct action of 17-methyl, 19-nortestosterone upon testis did not appear to occur since its administration did not inhibit HCG stimulation of testis in men with hypogonadal function (Harvey and Clemont, 1962).

Ericsson et al. (1964) reported inhibitory effects of progesterone and 6-chloro Δ^6-17-acetoxy progesterone (CAP) on the spermatogenesis in rabbit.

Macleod (1965) studied the effect of 6-Δ-medroxyprogesterone acetate (Depo Provera) on spermatogenesis. He reported that in one group a single injection of 1 gm resulted in azoospermia, 50-70 days after treatment, the effect lasting about 100 days after which recovery began. No feminization or loss of libido was observed.

Karkun and Kar (1965) administered megestrol acetate (40 mg/kg/day) by oral route and reported no effect on spermatogenesis or fertility of young and
mature rats. However, weights of the reproductive organs were reduced and gonadotrophin levels raised.

Kar et al. (1967) studied the effect of a number of progestational steroids on spermatogenesis in rats but only three viz. progesterone, 17α-hydroxyprogrenalone and Δ⁴-pregnene-3,20-dione were found to be active. Of these, 17α-hydroxyprogrenalone arrested spermatogenesis selectively without disturbing the androgenic function of the testis. Progesterone caused spermatogenic arrest at spermatocyte stage. All the 3 steroids did not act via pituitary but directly on testis.

Setty and Kar (1967) reported that percutaneous application of estrogens (viz. stilbestrol, estrone and 3-cyclophenyl ether of estradiol-17β) and progestagens (progesterone, 17α-ethyl-17β-hydroxy-5(10)estren-3 one, 17α-ethyl-3,17-dihydroxy 4-estrendiacetate and 16α, 17α-dihydroxy progesterone acetophenoxide) daily for 30 days effectively inhibited spermatogenesis. According to these workers, the effect might be due to a suppression of gonadotrophin levels.

Kamboj and Kar (1969) demonstrated the effect of progesterone on the biochemical composition of rat
seminiferous tubules. They further reported a decrease in fructose concentration accompanied by weight loss of accessory sex glands which was probably due to antiandrogenicity of the steroid at the level of pituitary-gonadal axis.

Progesterone, at a dose of 1-2.5 mg, for 30 days caused an increase in protein, nitrogen, ascorbic acid, glycogen and total lipid concentration of the seminiferous tubules but no change was recorded in the hyaluronidase activity and oxygen consumption rate (Jehan et al., 1970).

Singh et al. (1970) reported reduced weights of accessory reproductive organs after administration of Depo Provera.

Norgestrel, when injected intramuscularly at a dose of 1 µg for 30 days did not cause any adverse effect on the testis, but the accessory gland weights were reduced notably (Verma, 1972).

Norgestrel (0.1 and 0.5 mg/rat/day intramuscularly) administered for 96 days, caused reversible arrest of spermatogenesis. The lower dose was ineffective in causing spermatogenic inhibition, but proved antispermatogenic after 48 and 96 days of administration. Similar effects after 30 and 48 days treatment with the higher dose of
Norgestrel were recorded and partial restoration of spermatogenesis was reported after 96 days. Weights of seminal vesicles and prostate were greatly reduced. After 30 days, androgen deficiency was produced, while after 48 or 96 days, the androgenicity of the steroid was restored as was manifested by the functional status of the genital accessories. Loss of libido was also observed (Singh et al., 1972). But these workers observed inconsistent results of this compound at higher doses in rhesus monkeys.

Fotherby et al. (1972) studied the effects of low doses of progestins—chlopropramide acetate (500 µg daily) and norgestrel (100 µg daily) to normal male volunteers for 7-15 weeks. No significant changes were reported by them.

According to Lacy et al. (1973), the administration of three steroids i.e. ethynyl estradiol (0.01 mg/100 gm body weight), Proviron (0.6 mg/100 gm body weight) and norethisterone acetate (0.6 mg/100 gm body weight) reduced the weight of testis, epididymis and seminal vesicles while spermatogenesis seemed uninterrupted.

Terner and MacLaughlin (1973) studied the effects of progestins and androgen on the germinal cells of rat testis.
Long term treatment with a progestin (0.5-2.0 mg/day) caused atrophy of secondary sex glands and resulted in inhibition of spermatogenesis also accompanied by a loss of libido which could, however, be prevented by concurrent administration of testosterone (10-100 μg/day).

Frick (1973) reported azoospermia following oral administration of norethindrone (25 mg/day) or megestrol acetate (30 mg/day) and testosterone (released by silastic capsules) for 8-12 weeks. No loss of libido was noticed.

Coutinho and Melo (1973) reported a marked reduction in sperm count and mortality without loss of libido in 8 fertile men bearing subdermal silastic implants of testosterone followed by 100 mg/week of norgestrienone (17β-ethynyl 17α-hydroxyester-4,9,11-triene-3-one) or 13 ethyl norgestrienone given orally.

According to Johanson and Nygren (1973) when testosterone was released by implants along with 25 mg of norethindrone given orally for 3 weeks, followed by norethindrone once a week, there was noticed a reduction in sperm count, motility, and plasma testosterone level. No loss of libido was however reported.
Administration of methyltestosterone and ethynyl estradiol in the form of tablets, inhibited spermatogenesis but there was noticed a concomitant loss of libido and potency (Micheal et al., 1974).

Kragt et al. (1975) administered a combination of Provera (300 µg) and testosterone (40 µg) per 100 gm body weight to male rats and observed no significant effect on the weights of accessory organs after 10 days of treatment although testicular weights were significantly reduced.

Norethindrone (20 mg) given orally, daily was inadequate to inhibit spermatogenesis but it did suppress gonadotrophin and endogenous androgen levels. Testosterone administration (4 subdermal capsules of 20 mg each) was inadequate to maintain normal circulating levels of testosterone but had little effect on libido or potency (Brenner et al., 1975).

Frick (1976) implanted 4-6 silastic capsules containing megestrol acetate and testosterone and observed a resultant suppression of spermatogenesis without loss of libido.

Melo and Coutinho (1977) studied the inhibition of spermatogenesis following monthly parenteral administration
Healthy volunteers were treated with 100 mg Provera and 250 mg testosterone enanthate in separate intramuscular injections at monthly intervals. They noted a marked drop in sperm count after 1-3 months, representing an incomplete suppression of spermatogenesis.

Alvarez et al. (1977) reported azoospermia with combined monthly injections of DMPA and TE in 2 doses - (1) 1000 mg DMPA + 250 mg TE for 2 months followed by 150 mg DMPA + 250 mg TE (2) 1000 mg DMPA + 250 mg TE initially followed by 300 mg DMPA + 250 mg TE monthly. The azoospermia continued for several months thereafter.

Frick et al. (1977) administered DMPA and TE to human males at uniform dosage with a high initial dose (100 or 150 mg DMPA + 100 or 250 mg TE). Sperm production was severely reduced. In the high dose level, DMPA was initially injected at a dose of 100 mg, followed by 150 mg/month. Spermatogenic arrest was observed without any serious side effects.

Both increased and decreased accessory organ weights had been reported following treatment with estradiol benzoate and estradiol-17β respectively by Meistrich et al. (1977) and Hunt et al. (1979).
Flickinger (1977 a,b) studied in detail the effect of combined therapy of progestin and androgen (Provera - 1 mg/100 gm body weight and testosterone propionate - 100 μg/100 gm body weight) for 16 weeks on the structure of the male reproductive tract. The testis and epididymis weights were reduced and spermatogenesis seemed arrested at late spermatid stage. The different epididymal segments showed varying changes in the epididymal epithelium.

DMPA (100/150 mg) and TE (200 mg) injected intramuscularly for 4 months caused suppression of gonadotrophins and reduced sperm counts to almost azoospermic levels in all men. No loss of libido was reported (Brenner et al., 1977).

Lee et al. (1979) reported that 80% volunteers achieved either oligospermia or azoospermia with DMPA in monthly doses of 200 mg or 400 mg + testosterone cypionate in monthly doses of 200 mg or 400 mg.

Sharma and Kanwar (1980) reported reversible arrest of spermatogenesis in albino rats after daily administration of methyltestosterone and norgestrel through intramuscular injections for 4 weeks. Though not much variation of serum androgen level was found, seminal vesicle and prostate weights were reduced.
Monthly injections of 200 mg of DMPA with 250 mg of testosterone cypionate induced azoospermia in 56% of the subjects after 6-15 weeks and sperm count in the remainder was reduced to less than 5 million/ml (Paulsen et al., 1980).

Oral doses of 5 and 10 mg of cyproterone acetate administered daily for 20 weeks reduced sperm density and motility. Concentration of acid phosphatase, sialic acid and glycerylphosphorylcholine in seminal plasma decreased while fructose did not change (Roy et al., 1976).

Long term treatment with large doses of cyproterone acetate (200 mg/day for 7 months) caused loss of germ cells, altered appearance of Sertoli cells and led to involution of Leydig cells (Re Micali et al., 1979).

Treatment of male rats with 1.5 mg/day of chlormadinone acetate for 4 weeks caused reduction in weights of testis, epididymis, seminal vesicle and prostate. Increase of phosphatases, decrease of succinic dehydrogenase, sialic acid, citric acid and fructose in testis, seminal vesicle and prostate respectively was observed by Kaur and Mangat (1979).

According to Neumann (1979), cyproterone acetate and chlormadinone acetate are potent progestogens with anti-gonadotrophic effects. But inhibition of spermatogenesis is
dose and species dependent. Medium doses affect spermiogenesis while with high doses, the tubules become totally depopulated except for spermatogonia and early spermatocytes besides Sertoli cells.

Cyproterone acetate, at one time was seriously regarded as a potential male contraceptive. In the medium dosage range (10-20 mg daily) it lowered the depth of sperm penetration into a column of mid cycle cervical mucus in vitro and perceptibly also reduced the sperm count in human semen, while significantly also depressing the levels of circulating testosterone and gonadotrophin (Koch et al., 1976). At a higher level (100 mg daily), it not only reduced the number of ejaculated sperm but also rendered them immotile within a few weeks (Neumann et al., 1976).

Petry et al. (1972) reported reversible suppression of spermatogenesis in 5 men treated with cyproterone acetate for 11-16 weeks without any apparent effects on libido, whereas treatment with higher doses was shown to lead to loss of libido and potency (Morse et al., 1973). Later studies indicated that significant suppression of serum gonadotrophin and testosterone levels with low doses was accompanied by only a modest decline in sperm count (Fogh et al., 1979; Wang and Yeung, 1980). The limited success as
antispermatogenic agent, accompanied by occurrence of unplanned pregnancies and some untoward side effects, led to the belief that cyproterone acetate would be unsuitable as a single-entity agent for long-term male contraception (Wang and Yeung, 1980).

In the rat, impairment of epididymal function by cyproterone acetate has been observed by Prasad et al. (1970) and Prasad and Rajalakshmi (1977). In organ cultures of rat epididymal tubules, cyproterone acetate or 17-methyl-β-nortestosterone suppressed the activating influence of 5α-dihydrotestosterone on the development of sperm fertilizing ability in the proximal part of corpus epididymis (Orgebin-Crist et al., 1975). There are also reports indicating that cyproterone acetate and other anti-androgens upset the cellular activity of accessory organs other than epididymis.

The male antifertility effect of the synthetic anabolic steroid-danazol, which is an orally administered analogue of ethinyl testosterone, has been found to reduce plasma testosterone by direct inhibition and by reducing LH secretion (Sherins et al., 1971; Skoglund and Paulsen, 1973). Skoglund and Paulsen (1973) reported that treatment with danazol alone led only to a modest reduction in sperm count, but in combination with testosterone esters (propionate or
eranthate), it produced severe oligospermia and in some cases azoospermia. Later, Uldicin et al. (1975) reported that 6 months of oral danazol treatment combined with monthly intramuscular injections of testosterone enanthate reduced sperm counts, and increased the percentage of abnormal and immature forms of spermatozoa in ejaculate. No changes in libido and liver function were observed. Sperm counts returned to normal after 3 months of discontinuation of treatment. Paulsen et al. (1980) recorded decreased sperm counts to oligospermic/azoospermic levels, following treatment with danazol and testosterone enanthate. There did not seem to be any effect on libido. Danazol had no progestational or estrogenic effects but possessed weak androgenic activity and associated anabolic properties.

Intranasal spray of estrogen/norethisterone, administered daily at a dose of 30 µg for 60 days to male monkeys produced a marked reduction in tubular diameter and denudation of the germ cells except spermatogonia and of course Sertoli cells. Similar treatment with progesterone had a less marked effect. Serum testosterone levels were reduced (Anand Kumar et al., 1980).

Implants containing norethisterone enanthate inserted into the epididymal fat pads were noted to bring about
drastic reduction in size and weight of testis, 6 weeks after insertion on the side bearing the progestin implant. The caput and cauda epididymis showed absence of spermatozoa while testis showed absence of secondary spermatocytes, spermatids and spermatozoa in the shrunken seminiferous tubules (Srivastava and Malaviya, 1980).

Das et al. (1980) compared the antifertility effects of 17-hydroxyprogesterone caproate (17 OHPC), cyproterone acetate and flutamide in male rats. After 4-6 months of treatment with 17 OHPC or flutamide, the weight of epididymis and epididymal sperm concentration fell even though the weights of seminal vesicle or prostate were not affected. None of the drugs, injected for one month (250 µg/day) had any effect on the weights of the reproductive organs. Fertility was not affected by treatment with flutamide; cyproterone acetate had marginal effects (17% sterile) and 17 OHPC rendered 60% males sterile.

Bain et al. (1980) administered 250 mg/month of TE along with IMPA administered orally 5-20 mg/day for 6 months and reported suppressed sperm production even though severe oligospermia or azoospermia were not observed.
Ewing et al. (1983 a, b) reported that testosterone-estradiol implants (releasing 100 μg and 0.5 μg/kg body weight respectively) in male monkeys for 13 months caused reduction in testis weight and spermatogenic arrest at primary or secondary spermatocyte stage. Seminal vesicle and prostate were slightly heavier than control but the histology of the former was unaltered. No side effects were observed.

Unilateral implantation of norethisterone capsule (releasing 17-22 μg/day) into the epididymal fat pad of adult rats, for six weeks, induced drastic impairment of functional anatomy of epididymis. Tubular elements were shrunken and the lumen was devoid of spermatozoa in the adjacent epididymis without any effect on the contralateral one (Srivastava, 1983).

According to Arlbarg (1983), spermatogenesis in rabbits was completely suppressed by weekly injections of DMPA (10 mg) and TE (5 mg) for 6 weeks, although fertility was not impaired during induced oligospermia as determined by artificial insemination. Besides reduction in sperm density, there was not much effect on sperm motility or morphology.
Daily oral administration of 1 mg/kg of cyproterone acetate (CA) with simultaneous administration of TE (2 mg/kg/15 days, intramuscularly) to langur monkeys for 3 months caused gradual decrease in sperm count (to azoospermia) and spermatozoal motility with simultaneous increase in the incidence of non-motile and abnormal sperms. Lohiya and Sharma (1983) concluded that CA and TE seem to inhibit spermatogenesis in testis as well as sperm maturation in the epididymis without altering androgenicity.

Esterified 19-nortestosterone, an anabolic steroid, was administered intramuscularly to 5 healthy male volunteers in doses of 100 mg/week for 3 weeks followed by 200 mg/week for further 10 weeks. Azoospermia was induced after 7-13 weeks and persisted for 4-14 weeks after cessation of treatment. Serum gonadotrophin and testosterone levels were reduced but libido and potency remained unaltered (Schumeyer et al., 1984).

Daily subcutaneous injections of TE (100 μg) and ethinyl estradiol (0.05 μg) for 72 days brought about certain degenerative changes in the epididymis that included complete loss of sperm by the end of treatment. Biochemical variations were also reported as a result of drug treatment (Kanwar and Hazurla, 1985).
Moudgal et al. (1985) observed the effect of intranasal spray of norethisterone or progesterone on serum testosterone and total sperm count in adult male bonnet monkeys. While the norethisterone spray resulted in a drastic decrease in serum testosterone levels and sperm count, the progesterone spray caused a decrease in total sperm count only.

Knuth et al. (1985) administered 19-nortestosterone-hexoxyphenylpropionate (200 mg/week i.m. for 7 weeks). Serum gonadotrophin and testosterone levels were considerably reduced and severe oligospermia or azoospermia was observed in 10 volunteers. No effect on libido or on liver enzymes was discerned.

LHRH and its analogues

The pituitary and testicular response to LHRH following a single intravenous administration of 25 μg or 100 μg, was studied in 13 normal men by de Kretser et al. (1975). Both doses increased the plasma testosterone level but only 100 μg dose brought about a significant increase. The 25 μg dose raised LH level but not FSH whereas the 100 μg dose increased both LH and FSH levels.
Weigelmann et al. (1977) studied three submaximal doses of D-Ser(TBU)\(^6\)GnRH (i.e. 1.25 µg and 2.5 µg daily and 5 µg every alternate day) for 10 days in three groups of 5 normal males each. The 2.5 µg dose level reduced gonadotrophin response to analogue administration without reduction in basal gonadotrophins. The 5 µg dose did not diminish gonadotrophin responses while 1.25 µg dose suppressed responsiveness to FSH.

Labrie et al. (1978) demonstrated the suppression of spermatogenesis in rats with twice a week administration of 100 µg of D-ala\(^6\)des.Gly.\(^{10}\)GnRH ethylamide. This effect was notable by changes in the gross organ weight as well as in histology of the organ. Two months of treatment resulted in complete suppression of spermatogenesis.

Degenerative changes in the seminiferous tubules could be noted after two weeks of treatment with LHRH analogue. By 8 weeks all the tubules showed advanced degenerative changes which included disappearance of all germ cells (Pellétrie et al., 1978). When this analogue was administered at a higher dose (1 µg every second day), by 4 weeks degeneration had advanced to the stage of disappearance of Sertoli cells. A 40% decrease in testis weight was noted.
LHRH ethylamide led to progressive decrease in the weights of testis, prostate and seminal vesicle with maximum inhibitory effect at 12 weeks (Cusan et al., 1979). LH and FSH levels in plasma were increased.

Daily administration of 100 µg of an LHRH agonist, WX-18481 produced significant weight loss of testis within 7 days and of seminal vesicle and ventral prostate within 14 days. Progressive disorganisation and degeneration of seminiferous tubules was also observed (Corbin et al., 1979). Serum LH and testosterone were elevated.

Berquist et al. (1979) reported that chronic administration of 5 µg daily of \([\text{D-Ser-Tyr}^6, \text{Phe}^9, \text{Nle}^{10}]\)-LHRH to men caused a decrease in serum testosterone and gonadotrophin levels but spermatogenesis and potency were unaffected.

Acute or chronic treatment of adult male rats with LHRH or its agonist led to a loss of testicular LH and prolactin receptors accompanied by decreased testis, seminal vesicle and prostate weights (Labrie et al., 1980). Chronic treatment with LHRH agonists leads to marked histological damage in the seminiferous tubules after 4 weeks of treatment.
Hillius et al. (1980) treated 4 healthy men with 5 μg daily of the LHRH agonist D-Ser(TEU)⁶-EA⁶-LHRH. Treatment for 17 weeks reduced the basal LH, FSH and testosterone levels but spermatogenesis and potency were unaffected.

Rivier and co-workers (1980, 1981) demonstrated the antitesticular effects of \[\text{Ac-dehydro-Pro}^1-\text{pCl-D-Phe}^2-\text{D-Trp}^3,6-\text{Nc(-Me-Leu}^7)\]-LHRH in male rats. Administration of 1 mg daily led to a dramatic decrease in serum LH and testosterone levels and a decrease in testicular weight. Seminiferous tubule diameter was reduced and spermatogenesis was arrested. Treatment with optimum doses of testosterone led to restoration of mating behaviour but not of fertility.

Heber and Swerdloff (1981) reported that GnRH analogue alone suppressed serum testosterone while increasing LH and FSH levels. These workers noted lowered sperm counts in rats treated with 200 μg/100 gm body weight of D-leu⁶-des-Gly NH₂⁴ GnRH ethylamide for 60 days. When testosterone was given along with GnRH analogue, there was observed greater decrease in intratesticular sperm count but no increase in serum LH.
Rabin et al. (1981) tested the antifertility effect of \( [\text{D-trp}^6-\text{Pro}^9-\text{N-ethylamide}] \)-LHRH on healthy male volunteers. In men receiving parenterally 50 µg daily of this drug, serum testosterone and LH and FSH levels were markedly reduced, sperm density was reduced by 70% or more and there was a significant fall in sperm motility. When this analog was given every 4th day, there was wide fluctuation in the gonadotrophins and androgen levels and a modest decline in sperm density.

Vickery (1981) reported that in the dog, chronic administration of LHRH agonists resulted in a rapid fall of serum testosterone levels leading to a simultaneous decline in prostate weight and secretory function and a fall in the volume of ejaculate. Inhibition of spermatogenesis was very rapid, but reversibility was almost complete 24 days after termination of treatment.

Labrie et al. (1981) reported that treatment with LHRH agonists produced an inhibiting effect on testicular prolactin receptor concentration and could cause a dramatic fall in testicular androgens. Degenerative cellular changes were induced in rat testis during long term administration.
Linde et al. (1981) studied the antifertility effects of D-Trp\textsuperscript{6}-Pro\textsuperscript{9}-N-ethylamide-LHRH in 8 normal men who received daily subcutaneous injections of this drug for 6-10 weeks. Plasma testosterone levels fell simultaneously in all as did serum gonadotrophins. Impotence developed in 5 men within 6-7 weeks of treatment. Sperm density and motility fell considerably after 7th week.

Detailed studies by Faure et al. (1981) on treatment with LHRH agonists in male cats showed decreased weights of testis, ventral prostate and seminal vesicles, marked loss of testicular LH receptors and lowered plasma testosterone concentrations - leading to inhibition of spermatogenesis.

The effects on pituitary-gonadal function of the potent GnRH agonist D-Trp\textsuperscript{6}-Pro\textsuperscript{9}-N-ethylamide-LHRH were studied by Doelle et al. (1982). They gave 50 µg of LHRH-A subcutaneously every 4th day for 10 weeks to 7 healthy men and observed a modest rise in serum LH and a fall in sperm density but concluded that treatment every 4th day is not a promising regimen to induce suppression of pituitary gonadal axis.
Daily administration of relatively large doses (25-100 µg) of \([\text{D-Ser-His}^6\text{Pro}^9\text{Nle}^3\text{LHRH}]\) to male monkeys led to pronounced decrease in pituitary responses but testicular steroidogenic response and spermatogenesis were generally unimpaired as observed by Akhtar \textit{et al.} (1982) and Resko \textit{et al.} (1982).

Akhtar \textit{et al.} (1983) studied the effect of s.c. infusion of D-Ser(Bu)\textsubscript{6}-ethylamide GnRH (2 µg/hr) in monkeys for 20 weeks with testosterone replacement in the last 7 weeks. They observed an initial rise and then a considerable fall in serum LH and testosterone. Testicular weight fell markedly and spermatogenesis was inhibited to azoospermic levels. Spontaneous and electro-stimulated ejaculatory activities were lost until testosterone replacement therapy was given.

The GnRH agonist Buserelin was administered s.c. for 12 weeks to 2 groups of normal men (Schurmeyer \textit{et al.}, 1984). One group of seven men received 118 ± 24 µg/day and a second group of 4 men received 230 ± 27 µg/day. After an initial rise, serum LH, FSH and testosterone declined. Androgen substitution with testosterone undecanoate (80-120 mg orally, daily) was initiated after complaints of decreased libido and potency. Sperm counts decreased considerably and despite
desensitization of pituitary and impaired testicular function, azoospermia did not occur.

Daily injections of 50 μg of LHRH agonist \[\text{\text{D-Try}^{6}\text{des-Gly-NH}_{2}}\text{LHRH ethylamide}\] to adult male dogs for 16 weeks resulted in testicular and prostatic weight reduction and decreased sperm density and inhibition of testicular steroidogenesis (Tremblay and Belanger, 1984). Atrophy of seminiferous tubules and azoospermia were observed. Degeneration of testis had reached the extent of disappearance of almost all germinal cells. Recovery was witnessed after 26 weeks.

Tremblay et al. (1984), in order to maintain libido in dogs treated with LHRH-A for 16 weeks (as reported earlier) administered testosterone (170 mg/day) by percutaneous absorption. Administration of testosterone did not restore spermatogenesis which had been inhibited by LHRH-A, but prostate weight and ejaculate volume returned to normal.

Patients of disseminated prostatic carcinoma received treatment of \((\text{D-Leu}^{6}\text{des-Gly-NH}_{2}\text{proethylamide})\) GnRH (1 mg/day for 1 year). The testes of treated patients revealed seminiferous tubules which were highly
fibrotic and atrophied with only Sertoli cells left in them. No spermatozoa in testis or epididymis were seen. Leydig cells were less in number and showed signs of degeneration. Rajfer et al. (1984) concluded that chronic administration of GnRH agonists may prove to be a suitable male contraceptive.

The potential of a GnRH antagonist to inhibit reproductive functions in male monkeys was studied by Weinbauer et al. (1984). Continuous infusion of 2 mg/day of \([N-A\alpha-D-Nal(2)^1, D-pcl-Phe^2, D-Trp^3, D-h Arg(E_T)^6, D-Ala^{16}]-GnRH\), via osmotic mini pumps for 9 weeks caused immediate and sustained reduction of serum LH and testosterone and led to azoospermia in 3 animals and sperm count was less than \(5 \times 10^6\) in the fourth. Severe atrophy of Leydig cells and seminiferous tubules was observed. Recovery of endocrine functions was seen within 2 weeks and testicular histology was restored 14-18 weeks after termination of the treatment.

Sundaram et al. (1984) reported that there was a species dependent effect of a potent LHRH antagonist \([A\alpha-D-Nal(2)^1-4FD-Phe^2, D-Trp^3, D-Arg^6]-LHRH\). A dose of 1250 \(\mu g/kg\) for 15 days caused significant decrease in serum LH and testosterone as well as in the weights of the testis, seminal vesicle and ventral prostate in rats.
but no significant effect was seen in rabbits. Histology of testis and epididymis was adversely affected in rats. A dose of 1450 µg/kg daily for 5 days decreased serum testosterone and inhibited testicular steroidogenesis in rats but not in mice. These workers concluded that of rats, mice, rabbits and monkeys, the most sensitive to LHRH-agonists are rats.

Doelle et al. (1983) treated men to 50 µg daily of [D-Trp⁶-Pro⁹-Net]⁻LHRH along with 100 mg of testosterone enanthate every week. There was observed reversible oligospermia without loss of libido.

Habenicht et al. (1985) recorded that a single injection (25 µg in 0.1 ml/animal s.c) of an LHRH agonist D-Trp⁶-Des.Gly¹⁰-N Me Leu⁷-Pro⁹-Net, caused severe damage to rat testis within 24 hours. Histology of testis revealed a partial disorganisation of germinal epithelium due to degenerative changes in Sertoli cells. Also giant cells containing spermatids and degenerating pachytene spermatocytes were observed. Serum LH and testosterone were not much affected.

Gossypol and other plant extracts

Qian et al. (1972) reported that gossypol administered orally at a dose of 60-70 mg/day for 5-6 weeks
caused a gradual increase in the percentage of non-motile spermatozoa in the ejaculate. Oligo-/azoospermia was soon reached in all 25 volunteers. Sperm motility fell significantly by the 2nd week which suggested gossypol action on epididymal and testicular spermatozoa. Recovery was reported 3 months after the cessation of treatment. Side effects reported were mild and reversible.

In early studies in China, 15-40 mg/kg of gossypol given orally 5 times a day for 2-4 weeks produced decapitated spermatozoa with sperm tails twisted. These workers reported the presence of spermatids and even of the spermatocytes in the cauda epididymis and vas deferens. Gossypol treated animals which became infertile, mated normally and recovered their fertility within 4 weeks after cessation of gossypol treatment (Dai et al., 1978).

The subjects treated with gossypol at an initial dose of 40 mg/day for 2 months followed by a maintenance dose of 150-220 mg/month, divided and taken once or twice a week, revealed non-motile, malformed and dead sperm in their semen. Exfoliated abnormal spermatids and spermatocytes were also seen in the semen. Azoospermia was also reported to be achieved (National Co-ordinating Group, 1978). Hypokalemia and transient elevation of SGPT and mild changes in ECG were also reported after gossypol treatment.
When rhesus monkeys were administered gossypol at a dose of 4 mg/kg for 2 years, spermatogenesis was completely inhibited in 2 of the 3 animals (Sang et al., 1980). In the 3rd animal too, the seminiferous tubules showed only a few normal spermatids and spermatozoa.

Liu et al. (1981) observed that gossypol, at an optimal dose of 20 mg/day, for 60-70 days followed by a maintenance dose of 40-50 mg/week, caused infertility in almost 99% of the cases. Changes in the sperm count and motility recorded were similar to those reported following a dose of 60-70 mg, except that it took longer for these manifestations to appear. Some reversible side effects including hypokalemic paralysis (0.75%) were observed. Recovery, except in 10% of the subjects who were azoospermic, took 6 months to 4.5 years, indicating irreversibility.

Gossypol acetic acid, given to male rats at a dose of 7.5 mg/rat/day (6 days a week) for 10 weeks rendered all vas deferens spermatozoa immotile (Kalla et al., 1982). Although body weight and that of accessory sex organs was not affected, partial damage to testicular seminiferous tubules was revealed. Single high dose did not induce significant changes except a fall in testicular ATPase.
activity, while intratesticular administration evoked only localized damage.

Shandilya et al. (1982) observed that in monkeys treated with 10 mg/kg/day of gossypol acetic acid, there was noticed a significant fall in sperm concentration and spermatozoal motility accompanied by a concomitant increase in the abnormal sperm number. Transient azoospermia was also recorded. No decrease in serum testosterone, however, was reported.

Coutinho (1982) made the first clinical trial on gossypol outside China by subjecting 8 men for 6-12 months to 20 mg daily oral dose for 4 months followed by 20 mg gossypol on alternate days. Reduction in sperm count resulted after 45 days and azoospermia could be achieved after 4 months. No change in semen volume or libido/potency was reported. Blood chemistry and testosterone levels remained normal.

Coulson (1983) found that in mice treated simultaneously with gossypol or its derivatives and androgen, gossypol inhibited sperm production and androgen prevented change in activity of secondary sex glands.
Various doses (10, 20 and 30 mg/kg/day) of gossypol were administered to rats for 2-11 weeks by Hoffer (1983). She found that the seminiferous tubules were damaged, exhibiting intraepithelial vacuoles, exfoliation and atrophy. Electron microscopic studies revealed large intracellular vacuoles in Sertoli cells as well as an overall decrease in cytoplasmic ground substance, endoplasmic reticulum and Golgi apparatus. Structural disorganisation, exclusively of mitochondrial sheath in stage 18 and 19 spermatids was also discernible.

Wichmann et al. (1983) recorded highly inhibited fructolysis and glycolysis of human sperm due to gossypol treatment. Profound disturbances of sperm energy metabolism induced by gossypol were also reflected by a striking fall of sperm ATP content.

Gossypol acetic acid (4 mg/day 5 days a week) suspended in Tonoferon tonic given to male bonnet monkeys orally for 3 months caused a marked reduction in sperm count/ejaculate and sperm motility, both of which returned to normal 8-10 weeks after cessation of the treatment. Citric acid and fructose in semen did not vary much from control (Kalla et al., 1984).

Oko and Hrudka (1984) found that the early action of gossypol was directed at the developing mid-piece,
particularly the mitochondrial sheath. The delayed action caused selective degeneration of germ cells in stages VII and VIII, a retention of step 19 spermatids and exfoliation of spermatids and spermatocytes. A marked atrophy of androgen dependent organs was also observed.

Changamma and Reddanna (1985) studied the effect of gossypol acetic acid and 5-thio-D-glucose on glycogen metabolism in rat testis. Decreased glycogen content was noted through accelerated glycogenolysis, even though testis glucose was reduced. Phosphorylase activity was elevated after both treatments. Stepped up operation of hexose diphosphate pathway was indicated by increased activity of fructose diphosphate aldolase.

According to Kalla et al. (1985), 3 months of administration of gossypol at a dose of 0.5 mg/kg/day to male bonnet monkeys, reduced the motility and density of spermatozoa in the epididymis. According to them, gossypol appeared to be a potent male antifertility agent without overt toxicity in primates.

Antifertility effects of gossypol on rat spermatogenesis were studied by Haider et al. (1985). Oral administration of gossypol for 30 or 60 days reportedly
produced a plug of denuded germ cells and Sertoli cells in the lumen of the seminal canal. Immature spermatozoa at different developmental stages were to be seen in the ejaculate and also various structural abnormalities were encountered in the spermatozoa. These effects varied with the length of treatment and were reversible.

Pearle et al. (1985) incubated mouse Leydig cells with gossypol, studied their viability in vitro. Basal and LH stimulated testosterone production was inhibited by gossypol. Cytotoxicity of gossypol, which had been reported by these workers, did not affect steroidogenesis.

Baccetti et al. (1986) reported the action of gossypol on rat germinal cells. Consistent integrity of the blood-testis barrier was revealed in the testis of adult rats given 25 mg/kg of gossypol acetic acid. Earliest damage appeared, to be on the spermatids in stage 18-19, which was observed on 14th day of treatment and consisted of mitochondrial swelling and cristae disorganisation.

Chronic administration of flower extract of Malva viscus Gonzatti (25-50 mg/day for 25 days) caused testicular lesions resulting in atrophy of spermatogenic elements in house rat and gerbil (Dixit, 1977). There was
also recorded a noticeable decrease in the weights of epididymis, seminal vesicle and prostate. Epididymal epithelium regressed and the epididymal lumen did not show spermatozoa after the completion of the above treatment.

Dixit et al. (1978b) administered M. viacus flower extract to dogs at a dose of 150 mg/kg/day for 8 weeks and reported atrophy of the seminiferous tubules. The tubules were devoid of spermatozoa the germinal epithelium appeared highly disorganised. Epididymis and vas deferens were also empty though androgen dependent activity of the epididymis seemingly was unaltered.

Antifertility effects of alkylating agents and Vinca rosea alkaloids were studied in the rat by Cooke et al. (1978). All the agents (i.e. cyclophosphamide, nitrogen mustard vincristine and vinblastine) impaired fertility. Spermatogenic arrest at spermatid stage was observed in some sections. Morbidity and mortality was much higher with alkylating agents than with Vinca rosea alkaloids for similar degree of infertility produced.

Dixit et al. (1978a) reported that oral administration of fruit extract of Momordica charantia Linn, (1.75 g/day for 60 days) caused testicular lesions and atrophy of seminiferous tubules.
Oral administration of *Calotropis procera* flower extract every alternate day for 30 days reduced the weight of testis, epididymis and seminal vesicle in the gerbils (Garg, 1979). Testicular necrosis and degenerative changes in the germinal as well as Sertoli cells were also reported.

The effects of treatment with embelin and *Vinca rosea* extract were observed in rats by Chauhan *et al.* (1979). These effects were reported to be time and dose dependent. Reduction in weights of testis and prostate indicate impaired functions of these organs as does rise in activity of phosphatases.

Purandare *et al.* (1979) reported that oral administration of powdered berries of *Embelia ribes* (100 mg/day for 3 months) to male bonnet monkeys adversely affected quality and quantity of semen. Higher doses had an inhibiting effect on spermatogenesis.

Antispermatogenic effects of flower extract of *Hibiscus rosa-sinensis* were reported by Singwi and Lall (1980) on the testis of a non-scrotal bat. Germ cells and sperms were highly depleted.
Verma et al. (1980) reported antifertility effects of alcoholic extract of *M. viscus* flower on male mice (50 mg/mouse/alternate day). After 15 doses testicular histological damage was observed whereas 30 doses caused complete arrest of spermatogenesis and a highly atrophied state of seminiferous tubules. There was considerable loss of testicular weight.

Joshi et al. (1984) also studied the effects of alcoholic extract of *M. viscus* flower on male mice. The mice were administered 50 mg of extract daily for 30 and 50 days. Testicular lesions produced after 50 days were more pronounced than those after 30 days showing dose dependent effects. Weight reduction in testis and mass atrophy of spermatogenic elements was observed.

According to Baijal and Mathur (1981), steroidal fraction of *Abrus precatorius* tended to reduce epididymial weight as well as sialic acid and glycogen levels indicating impaired secretory activity.

Chronic administration of *Sapindus trifoliatus* fruit extract, to male gerbils (10 mg/animal/every alternate day) caused testicular lesions and inhibited spermatogenesis at primary spermatocyte stage (Dixit and Gupta, 1982a).
Weight of testis and epididymis was greatly reduced as was epithelial height of caput and cauda epididymis. Stereocilia were scanty and lumen devoid of spermatozoa.

Antispermatic/antiandrogenic properties of solasodine (from *Solanum xanthocarpum* berries) on male genital tract of dogs have been reported by Dixit and Gupta (1982). Administration of 20 mg/kg/every alternate day for 30 days, resulted in testicular lesions and impairment of spermaticogenic elements. Reduced levels of sialic acid in testis and epididymis and reduced Leydig cell nuclei reflected decreased androgen production.

Reversible contraceptive type activity of Embelin in dogs was reported by Dixit and Bhargava (1983). A dose of 80 mg/kg/alternate day for 100 days resulted in decreased weight of testis and epididymis, decrease of spermaticogenic elements in seminiferous tubules was observed along with empty epididymal lumen. 250 days of recovery brought back to normal the altered state.

Plumbagin, isolated from roots of *Plumbago zeylenica*, at a dose of 10 mg/kg intraperitoneally for 60 days caused selective testicular lesions in dogs (Bhargava, 1984) and reduced weight of testis and epididymis, shrinkage of
seminiferous tubules and reduced Leydig cell nuclei indicated sensitivity of androgen dependent structures to plumbagin treatment.

Pakrashi et al. (1985) reported the effect of M. viscosa flower extract on fertility of male rats. The extract given at a dose of 800 mg/kg for 30 days, reduced sperm count and motility and caused shrinkage of seminiferous tubules and reduced number of spermatids per tubule.

Antifertility effects of Solasodine (Solanum xanthocarpon fruit extract) was studied by Gupta et al. (1986) on male rats and dogs. No significant changes were recorded in weights of male reproductive and accessory organs but spermatogenic arrest at spermatid stage in rats and spermatocyte stage in dogs was observed. Sperm motility was also inhibited.

Dixit (1986) found that administration of Solasodine (20 mg/kg/day orally for 60 days) rendered male rats and dogs infertile. Testis and accessory reproductive organs did not exhibit weight changes, but inhibition of spermatogenesis and sperm motility was observed.
\(\alpha\)-Chlorohydrin and other chlorinated sugars

According to Samojlik and Chang (1970) sterility in male rats was observed after s.c. injections (1.5 mg/kg daily for 6 days and 40 mg/kg for 3 days) of 3-chloro-1,2-propanediol (U-5897). Higher dose for 7 or 20 days induced morphological changes in caput and cauda epididymis. Testis revealed complete inhibition of spermatogenesis. Recovery was not observed 140 days post-treatment.

Ericsson (1970) and Ericsson et al. (1971) found that lesions in the caput epididymis resulted after administration of daily oral doses of 35 mg/kg of \(\alpha\)-chlorohydrin for 8 days or a single dose of 45 mg/kg. Lesions were caused by sperm blockage in ductuli efferentes which in turn caused testicular swelling resulting in pressure necrosis.

Gilmore (1973) injected rats of proven fertility with triethylenemelamine (0.01 mg/kg), ethylene 1,2-dimethane sulphonate (10 mg/kg) and \(\alpha\)-chlorohydrin (1.5 mg/kg) for 25 days alone or in combination doses. Fertility of these animals dropped to a low level. Histological damage was restricted to post-meiotic stages of sperm production in testis and absence of spermatozoa in the epididymal tubules.
Hoffer et al. (1973) observed that single high doses (140 mg/kg) of \(\alpha\)-chlorohydrin produced histological lesions in the caput epididymis. These lesions were characterised by sloughing of epithelium, leading to obstruction of the lumen of the epididymal duct, spermatocele and sperm granuloma formation and ultimately to occlusive fibrosis. Within 48 hours the lumen was filled with degenerating cells that block further passage of sperm.

Kreider and Dutt (1973), treated mature rams with 25 mg/kg of \(\alpha\)-chlorohydrin for 18 days and observed the effect on accessory sex glands secretions. The composition of accessory gland secretion was not much altered, yet the motility and oxygen consumption of spermatozoa was much reduced.

Lubicz-Nawrocki and Chang (1974) injected male hamsters with \(\alpha\)-chlorohydrin at a dose of 66 mg/kg daily. There was noticed a decline in fertilizing ability of spermatozoa after 2 days and complete infertility resulted after 4 days.

Cooper et al. (1974) observed the antifertility effects of \(\alpha\)-chlorohydrin, \(\alpha\)-bromohydrin, epi-chlorohydrin and glycidol on the reproductive tract of male rats. \(\alpha\)-bromohydrin induced bilateral spermatoceles and permanent
sterility in rats but did not produce functional epididymal sterilizing effect of \( \alpha \)-chlorohydrin. Glycidol and epi-chlorohydrin showed effects similar to \( \alpha \)-chlorohydrin but the former did not cause lesions in the caput epididymis.

Brown-Woodman et al. (1974) found that spermatozoal motility as well as sperm concentration decreased when \( \alpha \)-chlorohydrin was injected at a dose of 25 mg/kg daily for 3 days to mature rams. Later, Brown-Woodman and White (1975) injected a single high dose (90 mg/kg) of \( \alpha \)-chlorohydrin to rats. After 48 and 240 hours of the injection, oxygen uptake and motility of sperm flushed from cauda epididymis was greatly reduced as was the amount of glucose utilised, glucose oxidised and lactic acid accumulated. They concluded that metabolic effects of \( \alpha \)-chlorohydrin were specifically confined to spermatozoa and this may account for reduced spermatozoal motility and fertility.

A single large dose (100 mg/kg) of \( \alpha \)-chlorohydrin administered to male rats increased capillary permeability in epididymis and induced vascular damage, sloughing of epididymal epithelium, occlusive fibrosis and formation of sperm granuloma and spermatocoele (Back et al., 1975).
Chronic administration to dogs of $\alpha$-chlorohydrin (8 mg/kg body wt. for 30 days) caused testicular lesions and degenerative changes in the germ cells. Epididymal cell height was reduced and lumen was devoid of spermatozoa. RNA and sialic acid contents of testis and epididymis were decreased, while total lipid and cholesterol were increased. These effects, reportedly were reversible (Dixit et al., 1975).

A single high dose of $\alpha$-chlorohydrin (70 mg/kg) caused pathological degeneration in dog testis after 33 days. Seminiferous tubules were depleted of spermatogenic elements and epididymal epithelium was regressed and lumen empty (Dixit and Lohiya, 1975). Dixit and Lohiya (1976) found that administration of $\alpha$-chlorohydrin to rats (25 mg/kg/day - 24 days), gerbils (20 mg/kg/day for 50 days), bats (75 mg/kg/day for 12 days), caused testicular lesions. Seminiferous epithelium became selectively depleted of spermatogenic elements but there was no direct effect on the epididymis. Growth of androgen dependent organs was suppressed. $\alpha$-chlorohydrin (30 mg/kg/day) did not cause any effect in testis and epididymis of mouse after 3-5 weeks.
A subtoxic dose of \( \alpha \)-chlorohydrin injected s.c. (10 mg/kg/for 30 days) to adult male langur monkeys caused reduction in the weights of testis and accessory sex organs (Braz et al., 1976). Spermatogenesis was suppressed but interstitial tissue appeared normal. Epididymal epithelial height was reduced. Sialic acid concentration in testis and epididymis and fructose in seminal vesicle and citric acid in prostate were also reduced.

Hunt et al. (1976) observed the effects of 17\( \beta \)-estradiol and Provera alone and in combination with \( \alpha \)-chlorohydrin in male rats. Fertility was not impaired by \( \alpha \)-chlorohydrin alone or in combination with Provera. Treatment with 17\( \beta \)-estradiol alone and in combination with \( \alpha \)-chlorohydrin caused infertility. After 25 days of treatment, spermatogenesis appeared arrested at spermatid stage, number of spermatozoa in epididymis were reduced and ventral prostate appeared devoid of secretory activity.

Kalla and Chohan (1976) administered a single oral dose (100 mg/kg) of \( \alpha \)-chlorohydrin to male rats. They reported desquamation of caput epithelium after 2 days of drug treatment. Blockage of caput epididymis appeared
because of accumulation of testicular fluid containing exfoliated cells. Spermatozoal morphology and motility were not affected.

Dixit (1976) observed that a single injection of α-chlorohydrin (20 mg) or cadmium chloride (0.04 m mole/kg) into the vas deferens of dogs affected testicular function adversely. Atrophy of testis reflected in the form of extensive necrosis and exfoliation of cells of seminiferous tubules were observed along with decrease in testicular weight and seminiferous tubule diameter after 4 weeks of drug administration.

Chronic administration to langur of α-chlorohydrin alone (140 mg/day for 40 days) caused testicular lesions resulting in massive atrophy of spermatogenic elements. Epididymal epithelium was regressed and lumen devoid of spermatozoa. A lower dose (1/4) of α-chlorohydrin too caused the same effects when administered with a gonadotrophin inhibitor - Methallibure. The latter alone had no damaging effects on testis or epididymis (Dixit, 1977).

Homm et al. (1977) reported that 5 weeks of daily treatment with 50 mg/kg of 5-thio-D-glucose to male rats caused early testicular degeneration with the presence of
spermatidial giant cells, spermatogonia and a few primary spermatocytes. Leydig cells were not affected and were the weights and histology of accessory reproductive organs.

According to Lobl and Porteous (1978) spermatogenesis and fertility was inhibited when 5-thio-D-glucose was administered orally to mice at a dose of 50 mg/kg/day for 49 days. Reversibility of antispermatogenic effect was only partial. Testis weight was reduced after 29 days and multinucleated giant cells, foamy vacuoles and amorphous masses were found. After 49 days, the tubules appeared highly shrunken and also showed vacuolisation and depletion of germinal cells. The antispermatogenic effect was found to be independent of diabetogenic effect.

Das and Yanagimach (1978) treated male hamsters with monothioglycerol (25 mg/kg/twice daily for 5 weeks), L-chlorohydrin (100 mg/kg for 1 week) and 5-thio-D-glucose (silastic implants). Monothioglycerol induced infertility, but when epididymal spermatozoa were artificially deposited in the uterus, fertilization took place so interfertility was probably due to sperm toxic action of accessory gland secretions rather than lack of fertilizing capacity. L-chlorohydrin induced sterility due to disease in fertility. There was recovery after 1 week of cessation.
In vitro incubation of sperm revealed a decrease in ATPase activity. No weight changes were observed. It was conjectured that there was an epididymal site of action of this compound or metabolites mediated through the glycolytic pathway.

Veeraragavan and Ramakrishnan (1984) reported that 33 mg/kg, for 21 days of 5-thio-D-glucose administered to mice affected testicular steroidogenic activity adversely. There was a decrease in steroidogenic as well as marker enzyme activity in the testis. Testicular histology revealed that all stages of spermatogenesis were affected in as much as most of the seminiferous tubules contained only a few spermatogonia amongst Sertoli cells.

Male rats given 6-chloro-6-deoxy-glucose (24 mg/kg, P<0.0) became infertile after 5 days and maintained at the same dosage level for 8 weeks with fertility restoration within 2 weeks (Ford and Waites, 1978). Since spermatogenesis or libido was not affected, the target site must be either epididymis or spermatozoa.

Lesions in the CNS and other CNS changes were observed after chlorosugar treatment to marmosets and mice (Jacobs and Duchen, 1980; Jacobs and Ford, 1981). These led to a decrease in the interest in chlorosugars as male antifertility agents.
Clomiphene citrate is another non-steroidal compound which has been used to treat male infertility at lower doses, whereas at higher doses, it is found to cause some degree of infertility.

Nelson and Patanelli (1962) who administered orally, clomiphene citrate to 30 days old male rats at doses varying from 0.1 - 25.0 mg/kg daily, weights of testis and accessory sex organs decreased at all doses above 0.25 mg/kg and were at hypophysectomy level above 2.5 mg/kg. Spermatogenesis halted at early spermatid stage as in hypophysectomised or estrogen treated rats. Pituitary gonadotrophin levels decreased at all doses. In adult males, doses of 25 mg/kg were necessary to produce effects similar to those in young males at lower doses.

Roy et al. (1964) found clomiphene to decrease testicular and accessory organ weights at higher doses (1 and 3 mg/kg for 34 days), but at very low doses (0.25 - 0.5 mg/kg/day), there was an increase in weights of seminal vesicle and prostate of immature rats.

Clomiphene, administered to rats at a dose of 250 μg/day for 25-55 days or 1 mg/day, inhibited spermatogenesis at primary spermatocyte stage, Leydig cells were
atrophic and consequently accessory glands non-secretory (Kalra and Prasad, 1967).

Dose-dependent action of clomiphene citrate was recorded by Heller et al. (1966, 1969). They administered daily, twice orally, clomiphene citrate to 14 normal men at doses of 50, 100, 200 and 400 mg/day for 2-12 months and observed increased excretion of total gonadotrophin and urinary ICSH throughout treatment. Sperm concentration was dose-dependent - low doses of clomiphene stimulated and high doses inhibited sperm number (Heller et al., 1969). Only one man became azoospermic with 400 mg after 2 months. But at all doses there was shrinkage in the mass of testis and hyalinization of tubular membrane. All cell types were present but 30-60% spermatids appeared damaged and sperm count fell significantly.

Clomiphene citrate (CC) treatment in men (100-200 mg/day for 6-9 days) stimulated the pituitary-Leydig cell axis causing an increase in plasma LH and testosterone levels (Bardin et al., 1967). Cargille et al. (1968) also showed increase in urinary excretion of FSH, LH in CC treated men showing a direct action of CC on hypothalamic-pituitary axis for release of LH.
Due to the dose-dependent stimulatory effects of CC on hypothalamic-pituitary axis, it has been used for clinical tests for evaluation of gonadotrophin reserve in hypogonadal patients with hypothalamic-pituitary disorders (Peterson et al., 1968). Baier et al. (1969) demonstrated a significant decrease in hypothalamic FSH-RH content with a corresponding increase of plasma FSH after 100-300 µg clomiphene injection in rat and thus confirmed the interference of this drug at hypothalamic level.

Kampmann et al. (1976) reported that clomiphene, besides having stimulatory effects on serum FSH, LH and testosterone also increased urinary DHT and estrogens in normal men. Subcutaneous injection of clomiphene resulted in an arrest of spermatogenesis, while intratesticular injection revealed more drastic changes viz., disappearance of seminiferous tubules and interstitial tissue. These phenomena indicated the suppression of pituitary gonadotrophin hormone, which might be due to the oestrogenic nature of this compound (Kaul and Ramaswami, 1968).

Epididymal function in rats seemed to be altered by administration of cis- and trans-isomers of clomiphene citrate at a dose of 500 µg/kg/day for 60 days (Rajalakshmi et al., 1970). Sialic acid content was reduced and there
was regression of epithelium, increase in intertubular tissue and pycnosis of nuclei.

Singh and Prasad (1973) observed that cis-clomiphene decreased only accessory organ weights, whereas trans-clomiphene reduced testis weight as well. Secretory activity of glands was reduced in both cases. Epididymal spermatozoa were non-motile at the end of treatment and early recovery. Fructose and citric acid levels were reduced.

Vitale et al. (1973) found that clomiphene also retards the development of blood-testis barrier when administered to young male rats.

Flickinger (1977c) treated young mature male rats with clomiphene citrate (2.5, 3.5 or 5.0 mg/100 g/day for up to 12 weeks). Accessory organ weights were found to be reduced; degree of reduction increased with the increase in dose and length of treatment. Longer intervals reduced testicular and epididymal weights also. Absence of late spermatids and accumulation of lipid droplets was observed. After 12 weeks more extensive changes were seen degeneration of primary spermatocytes and in some seminiferous tubules only Sertoli cells with a few
spermatogonia. Epididymal lumen was devoid of spermatozoa and Leydig cell size was reduced.

Chan et al. (1985) studied the effects of CC at various concentrations (0.005, 0.05, 5, 50 µg/ml) on in vitro motility and fertilizing capacity of human spermatozoa. They found that CC decreased fertilizing capacity in vitro which was probably due to sperm immobilizing activity of CC.