CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

PART I

Infertility is a common problem that affects more than 10% of all married couples. Couples are medically defined as being infertile if one year of unprotected intercourse has not resulted in pregnancy. The female partner is responsible for more than 60% of infertility.

The reproductive function in human beings result from the complex interaction between the hypothalamus, pituitary, and gonads. The hypothalamic-pituitary axis basically controls reproductive function through a series of neurohormonal secretion which cause the secretion of follicle stimulating hormone (FSH) and Luteinizing hormone (LH), the gonadotropins which acts on the target organs, the gonads.

Hormonal factors play a key role in the control and regulation of reproduction, each event of which is under the control of spontaneous and precisely timed interplay. The hormones primarily concerned with reproduction include those of the hypothalamus, pituitary, gonads as well as thyroid, adrenal and pineal glands.
HORMONAL REGULATION IN THE FEMALE

The ovulatory cycle upon which fertility depends is regulated by various internal as well as external factors, i.e. hormone secretion, light, smell, temperature, stress, metabolic alteration, emotion, etc. It was believed that there are two major sites of action within the brain which are playing an important role in reproduction viz., the hypothalamus and the pituitary gland. However, recent developments suggest that the complex sequence of events known as the menstrual cycle is controlled by the sex steroids produced within the follicle destined to ovulate (Speroff et al., 1989).

Reproductive activity in the female is characterised by the menstrual cycle which is divided into 3 major phases.

1. Follicular phase.
2. Ovulation or Ovulatory phase.
3. Menstruation or Menstrual phase.

Each phase is characterised by precise endocrine modifications which are the result of secretory process of endocrine glands.

FOLLICULAR PHASE

The initiation of follicular growth appears to be independent of gonadotropin stimulation. The action of FSH in the female involves activation of granulosa cells and their development. Specific receptors for FSH are present on preantral
granulosa cells and in the presence of FSH, the preantral follicle can aromatize limited amount of androgens and generate its own estrogenic micro-environment (Mc Natty, 1979). Estrogen production is therefore, also limited by FSH receptor content.

The role of androgen in follicular development is complex. When exposed to an androgen rich environment, preantral granulosa cells favour the conversion of androstenedione to the more potent 5α reduced androgens rather than estrogens (McNatty, 1979). These androgens cannot be converted to estrogen and in fact, inhibit aromatase activity (Hillier, 1980). They also inhibit FSH incubation of luteinizing hormone (LH) receptor formation, another essential step in follicular development (Jia, 1985). The fate of the preantral follicle is in delicate balance. At low concentration, androgen enhances their own aromatization and contribute to estrogen production. At higher levels, the limited capacity of aromatization is overwhelmed and the follicle becomes androgenic and ultimately atretic (Erickson et al., 1985). Therefore, perhaps follicle will progress in development only if emerging when FSH is elevated and LH is low. The success of a follicle depends upon its ability to convert an androgen microenvironment to an estrogen microenvironment (Chabab et al., 1986). The growing sensitivity of FSH and LH which is brought about by the action of FSH and the enhancing influence of estrogen are responsible for conversion from an androgen microenvironment to estrogen microenvironment.

At the mid follicular phase, FSH is gradually decreased by changing its mode of action. FSH induces LH receptor development on the granulosa cells of
the large antral follicles. With increasing concentration of estrogen, within the follicle, FSH induces LH receptor instead of stimulating its own. The combination of a capacity for continued response despite of decreasing levels of FSH and a high local estrogen environment in the dominant follicle provides optimal conditions for LH receptor development.

Approaching maturity, the preovulatory follicle produces increasing amount of estrogen, during the late follicular phase, estrogen rises slowly at first then rapidly reaching a peak approximately 24-36 hours prior to ovulation (Pauerstein et al., 1978).

During late follicular phase, LH promotes luteinization of the granulosa in the dominant follicle, which results in progesterone production. This small but significant increase in progesterone production has its own importance to affect the positive feedback response to estrogen which induces a characteristic LH surge (Collins and Hodgen, 1986; March et al., 1981).

Although prolactin is always present in the follicular fluid, there is no evidence to suggest that prolactin is important during ovulatory cycles in the primates (Speroff et al., 1989).

OVULATION

The onset of the LH surge appears to be the most reliable indicator of impending ovulation, occurring 34-36 hours prior to follicle rupture (Hoff et al., 1983). The LH surge continuously rising up in the follicle indicates the resumption of meiosis in the oocytes, luteinizing of granulosa cells and synthesis of
prostaglandins, which are essential for follicle rupture. The mechanism by which prostaglandins induce follicle rupture is still not clear.

Along with the LH surge, levels of progesterone in the follicle continue to rise till ovulation time and the continuously rising progesterone levels might act to terminate the LH surge as a negative feedback. The low midcycle levels of progesterone exert an inhibitory action on further granulosa cell multiplication and the drop in estrogen may also reflect this local follicular role for progesterone (Speroff et al., 1989). Progesterone enhances the activity of proteolytic enzyme responsible together with prostaglandins, for digestion and rupture of the follicular wall.

LUTEAL PHASE

After ovulation, the follicle is known as corpus luteum whose life span and steroidogenic capacity are dependant on continued tonic LH secretion.

Normal luteal function requires optimal preovulatory follicular development. Suppression of FSH during the follicular phase is associated with lower preovulatory estradiol levels, depressed midluteal progesterone production and a decrease in luteal cell mass (Smith et al., 1985).

Progesterone levels normally rise after ovulation for 8 days after the LH surge, decreasing initial levels 24 hours before onset of the next menstruation (Ross et al., 1981).

Progesterone is the principle hormone in the maintenance of pregnancy and acts on the alveoli of the mammary glands to stimulate its function.
A complex interaction and interplay exists between the various hormones regulating the menstrual cycle. Specific sequential changes in the response of granulosa cells to gonadotropins appear to be related in part, to hormone specific regulation of hormone receptors. The interference with gonadotropin and the low estrogeneric steroid secretion would result in clinical aberration within the menstrual cycle inducing infertility.

CAUSES OF INFERTILITY

Various factors play a key role to cause infertility in females i.e. body weight, hormone imbalance, chromosomal abnormalities, environmental changes, stress condition, metabolism etc., as discussed below.

BODY WEIGHT AND INFERTILITY

Body weight plays an important role in female infertility. Obesity and weight loss are correlated with hormone imbalance which lead to infertility or amenorrhea in females. According to Mason et al. (1988), weight loss in women results in a hypothalamic disturbance of gonadotropin regulation with reduced frequency of luteinizing hormone pulses in women related to amenorrhea or multifollicular ovaries. Moreover, Green et al. (1988) also reported in their study that women with 15% or more below their ideal body weight were unable to conceive and had a 4.7 fold increased risk of infertility associated with ovulatory dysfunction. Weight loss, in its extreme form of anorexia nervosa is associated with hypogonadotrophic hypogonadism (Speroff et al., 1983). Sometimes
polycystic ovarian syndrome (PCOS) could be related with body weight. According to Fox et al. (1991), PCOS and estrogen deficiency without hirsuitism might be related with weight loss and hyperprolactinemia the precipitating cause of amenorrhea. In contrast, Rojanasukul et al. (1988) and Wild and Bartholomew (1988) reported that PCOS is correlated with weight gain in women with abnormalities in serum lipid and lipoprotein levels.

METABOLISM AND INFERTILITY

Metabolic abnormalities have often been related with PCOS which could be the cause of infertility in PCOS women. Hyperandrogenic state has been blamed to create abnormalities in lipoprotein as well as carbohydrate metabolism. Wild and Bartholomew (1988) have reported that women with polycystic ovarian syndrome have been shown to have lipoprotein profiles closer to those of men, than to regularly menstruating women. Evidence was also available that testosterone and other androgens are regulating factors of high density lipoprotein (HDL) and cholesterol. The androgen status adversely affects the high density lipoprotein/low density lipoprotein (HDL/LDL)/cholesterol balance and the body weight appears to affect triglyceride levels.

HORMONES AND INFERTILITY

The studies made in reproductive endocrinology during the past decades may attribute partly to enhanced awareness of the interrelationship among the hypothalamus, anterior lobe of the pituitary gland and ovaries. Although the
pituitary gland and hypothalamus have long been recognised as closely related anatomically and physiologically, only recently enough evidence has been accumulated to establish neurohormone as the link between the two structures. Further, biochemical events within the brain involving synthesis of biogenic amines including norepinephrine, serotonin and dopamine are known to regulate hypothalamic function. The stimulatory and inhibitory effects of biogenic amines may serve as the link between environment (internal and external) and hypothalamic control over pituitary. Study of the physiology of GnRH in human beings is limited by difficulty in measuring the peptide directly in the peripheral circulation due to its relatively complete confinement within the hypophysial portal blood supply as well as its short circulating hypophysial life (Arimura et al., 1974). However, the key concept is that normal menstrual function requires pulsatile secretion of GnRH and its amplitude (Gross et al., 1985; Reame et al., 1984; Mais et al., 1986).

The ovulatory menstrual cycle of the female involves a dynamic sequence of changes in hypothalamic pituitary ovarian axis. Maxson and Weintz (1983) reported that the most accurate documentation of ovarian failure is the detection of gonadotropin secretion.

Failure of the ovarian follicular apparatus results in decline of estradiol and ovarian peptide (particularly inhibin) which normally exerts a negative feedback supression on the hypothalamic-pituitary axis. Secretion of biologically inactive FSH as well as the presence of antigonadotropic antibodies have been excluded as causes. The significant greater elevation of FSH than LH suggests the possibilities
that the ovaries are not producing inhibin, which has a specific restraining effect on FSH secretion.

Moreover, McDonough (1978) had shown that primary ovarian failure or primary amenorrhea in women is related with hyper gonadotropic hypogonadism. Some authors also reported that a complete absence of detectable GnRH secretion was observed which is supported by Speroff et al. (1989) and classified as hypothalamic amenorrhea.

In case of polycystic ovarian syndrome, changes in pituitary gonadotropin may be the main cause of the disease in women, particularly elevated level of serum LH (Yen et al., 1970).

In the diagnosis of primary ovarian insufficiency, the plasma estradiol level often seems to be low in conjunction with elevated LH and FSH. The FSH level exceeds the LH level so that the LH to FSH ratio decreased in primary ovarian failure.

A deficiency of gonadotropin production can result from either congenital or acquired hypothalamic or pituitary disorders. Conditions producing hypogonadotropism due to primary involvement of the hypothalamic pituitary axis are usually associated with hypoestrogenism. Moreover, earlier in our laboratory it was observed that primary amenorrhea could be due to primary ovarian failure (POF) with elevated gonadotropin level and low estradiol levels. As well as it was also found that hypofunction of hypothalmo pituitary axis (low level of gonadotropin hormones with low estradiol) was also responsible for primary amenorrheic condition in females (Pandya, 1994).
Hypersecretion of prolactin is the most common pituitary disorder seen in clinical practice. Hyperprolactinemia is the common cause of amenorrhea, galactorrhea and infertility and is estimated to occur in one-third of women with no obvious causes of amenorrhea. The frequency of hyperprolactinemia in PCOS has been reported to be between 13% to 41% (Futterweit and Krieger, 1979; Jaffe et al., 1978; Wortsman and Hirschowitz, 1980; Falaschi et al., 1980; Lunde, 1981).

The clinical presentation of hyperprolactinemia is well known and usually takes the form of reproductive dysfunction. In women, primary or secondary amenorrhea is the most common symptom but irregular menses or infertility related to an inadequate luteal phase may also be the presenting complaint of elevation in prolactin levels. Amenorrhea is also found associated with altered level of prolactin which inhibits the pulsatile secretion of GnRH. Throughout the luteal phase, progesterone is the principle secretory product of corpus luteum although substantial amount of estrogen and other steroids are also secreted (Ross et al., 1970). Pandya (1994) also found that hyperprolactinemic hypogonadism was detected in patients where the serum FSH level was very low, LH was not detectable and prolactin levels were thrice the normal levels in primary amenorrheic women.

Deficiency in either the duration of progesterone secretion or the amount secreted during the post ovulatory interval of the menstrual cycle have been widely correlated with reproductive failure (Mozkowski et al., 1962; Ross et al., 1970; Sherman and Korenman, 1974a,b; Strott et al., 1970; Jones and Marigal-Castro, 1970; Weintz, 1979; Landgren et al., 1980). Moreover, according to Weintz (1980)
and Rosenfeld and Garcia (1976), deficiency in progestrone by the corpus luteum lead to inappropriate endometrial development so that normal nidation is impaired or prevented.

Patients with amenorrhea with androgen overproduction related to adrenal or ovary, may occur as a result of a virilizing adrenal tumor or cogenital adrenal hyperplasia. While the principle cause of ovarian androgen production is the androgenic ovarian syndrome or polycystic ovaries (McDonough, 1978).

Patients with primary hypothyriodism and hyper-prolactinemia can present with either primary or secondary amenorrhea. Thyroid hormones, T3 and T4 as well as TSH could also affect metabolism of gonadotropin hormones in case of hypothyroidism or hyperthyroidism. An altered serum cholesterol level is frequently associated with disordered thyroid function. Taskinen and Nikiila (1988) studied that in hypothyroid women, during the course of thyroxine treatment, a significant increase in sex hormone binding globulin level occurred as thyroid hormone level normalised.

As mentioned earlier, body weight (Obesity, Weight loss) and metabolic disorder play a key role in irregular cyclicity and fertility in the female. The thyroid gland is the major control centre of these metabolic regulations. Evaluation of the thyroid status is essential therefore, to determine weight and metabolism related factors which may contribute to female infertility.

INFECTION AND INFERTILITY

Infertility is well recognised as an important sequelae of genital tract
infection. Of major concern is the current epidemic of sexually transmitted diseases (STD). According to a report of The Times of India (The Times of India, 2nd Feb., 1995) atleast 40% of the population, predominantly women of underprivileged classes from Maharashtra State in India, who suffer from STDs, do not get medical attention. They are potential victims of infertility or HIV infection. Moreover, STDs is associated with secondary epidemic of acute salpingitis, tertiary epidemic of tubal obstruction. It has been suggested that certain sexually transmitted pathogens may directly contribute to infertility or reproductive wastage (Sweet, 1988). The genital tract mycoplasmas (M hominis and V urealyticum) are the cause of a variety of genital tract infections including post-partum fever (Wallace et al., 1978), acute salpingitis (Mardh and Westrom,1970), non-gonococcal urethritis (Lee et al., 1986; Taylor et al., 1980) and chorioamnionitis (Shurin et al., 1975) and continue to be implicated in infertility, spontaneous abortion, stillbirth and low birth weight.

IMMUNOLOGY AND INFERTILITY

It is theoretically possible for autoimmunity (in the male) and isoimmunity (in the female) directed against a variety of reproductive tract antigens to cause infertility (Fones, 1986). However, Alper and Garner (1985) showed that women with premature ovarian failure often were associated with autoimmune disorder which was found in 39% of female patients. Moreover, they also suggested that thyroid disease in fact may be the most common autoimmune-disorder found in patients with premature ovarian failure. The large number of disorders of the
immune system that are associated with premature ovarian failure strongly suggest that many patients develop premature menopause through an auto-immune mechanism.

CHROMOSOME CHANGES AND INFERTILITY

Alteration in the structure and number of sex chromosomes are frequently responsible for sex abnormalities, in the dysgenesis of the foetal gonads leading to endocrine imbalance and phenotypic abnormalities. Detection of structural abnormalities, deletion, inversions and translocations have been related with poor pubertal development and absence of menstruation. Patients with primary amenorrhea or abnormalities of pubertal sequencing frequently have impaired gonadal function (McDonough, 1978; Reindollar et al., 1981) Varying degrees of gonadal differentiation in relation to sex chromosomal anomalies, including non mosaic (45,X) and mosacism (45,X/46,XY; 45,X/46,XX; 45,X/46,X; (Xq) and 45,X/46,XX; del XX; 46,X,iso (Xq); 46,XX/46XY, 46, XY female, 46,XX male, 46,XXY male have been discussed by McDonough (1978), Sheth et al. (1988) and Shah et al. (1990). Moreover, a case report was described by Sheth et al. (1994) showing that a patient with 46,X idic (Xq) karyotype had short stature lack of axillary and pubic hair, under developed secondary sex characters, with elevated levels of FSH and LH with low E2 suggested primary ovarian failure which could be due to chromosomal anomalies in the individual. Also, Radhakrishna et al. (1991) have reported a triple-X female with long arm deletion of one of the X-chromosomes 47,XX, +del (X) (q27.3) associated with primary amenorrhea with
normal prolactin, elevated levels of gonadotropin with low estradiol and progesterone which might be first report of a triple X with deletion of X chromosome associated with primary amenorrhea. Cytogenetic aberration leading to sex abnormalities and infertility have been studied in great detail (Simpson, 1982). The deletion of a chromosome or part of a chromosome results in loss of genetic information, influencing indirectly the endocrine function and differentiation patterns.

CHROMOSOMAL ABNORMALITIES IN FEMALES

Turner’s Syndrome

The genetic causes of ovarian failure, or Turner’s Syndrome is associated with the absence of one X chromosome (45,X). This chromosomal abnormality leads to short stature, undeveloped reproductive system, absence of menarche etc. (Shah et al. 1989).

Testicular Feminization Syndrome

Occasionally, phenotypic females diagnosed as having primary amenorrhea have male karyotype 46,XY, a sex disorder resulting in complete or incomplete testicular feminization. Testicular feminization arises as an X-linked recessive gene defect and results in insensitivity of all androgen target organs to testosterone. Some reports are also available on hormonal profile of the testicular feminization syndrome (Portuondo et al., 1984). With recent advances in endocrinology and genetics alongwith the increased use of laparoscopy and ultra sonography, it has
become apparent that a spectrum of disorders exist and that delineation of these disorders on an anatomic or chromosomal basis alone is inadequate to explain the cause of each anomaly.

A case report by Sloan et al. (1984) has shown that a rare dicentric Y chromosome was found in primary amenorrhea and virilization in a true hermaphrodite girl of 24 years old, leading to infertility in this patient with abnormal hormonal profile.

ENVIRONMENTAL FACTORS, TOXICITY AND INFERTILITY

Environmental factors play a key role to cause infertility in men as well as in women. Temperature, light, smell etc., are responsible for infertility. Moreover, certain environmental agents viz., fluoride, mercury and lead can also lead to impaired fertility in men as well as in women. In our laboratory it was reported that fluoride can impair fertility in both, male and female rodents (Chinoy and Sequeira, 1989a,b; 1992; Chinoy, 1991a,b; 1992; Chinoy et al., 1993; 1994a,b; Narayana and Chinoy, 1994a,b). Moreover, fluoride can affect the motility of human spermatozoa as well as the reproductive system (Chinoy, 1992). Lead is also known to cause reproductive impairment and growth retardation in male rats as well as in human beings (Rao et al., 1987; Thakkar et al., 1993).

STRESS, SOCIAL STATUS AND INFERTILITY

In a socially orthodox society such as ours, with traditional emphasis on child bearing, anovulatory infertility represents a social stigma. Therefore, the role
of emotions is one of the most controversial issue in infertility treatment. Although psychological stress factors have been recognised as capable of producing amenorrhea (Seward et al., 1965; Fries et al., 1974; McDonough, 1978) such couples suffer extreme social pressure, frustration and anxiety. When such cases are neglected in the course of investigation or inadequately counselled, its consequences are emotional anxiety, marital stress and instability.

Therefore, the present study was carried out to investigate causes of infertility from Ahmedabad city, various regions of Gujarat and its neighbouring vicinity.

A special attempt was made in the work carried out in this thesis, to investigate various biochemical as well as endocrinological factors correlated with gynatresia if any, with the help of ultrasound scanner as well as chromosomal analysis (wherever necessary) in females suffering from infertility, in the light of earlier work.
The growth of human population has assumed alarming proportions and the necessity of ensuring adequate food, clothing, housing and health has become a herculean task. The People and Governments especially in developing countries are forced to look for safe, easy and effective ways to control fertility. Therefore, today for achieving world’s stable population, research in fertility regulation has been given top priority.

The history of fertility control could be traced back to 4000 years, with the discovery of a precipitation for contraception written on an ancient Egyptian papyrus. One of the methods suggested in the papyrus was the local use of paste containing grounded accacia (Havemann, 1967).

Various methods have been developed and are being devised to control the population and a number of contraceptive methods are now available to combat the rapid growth of population. These different methods have varying degrees of failure rate. The methods include oral contraceptives, paper pills, steroid combinations, copper and medicated IUDs, vaginal rings (with or without steroids), vaginal barriers contraceptive, cervical-vaginal subdermal implants, sterilization, immunological methods, anti-fertility drugs, plant alkaloids, prostaglandins and other abortifacient agents, spermicidlals, nasal spray, biodegradable implants, etc.
DIFFERENT TYPES OF CONTRACEPTIVES

BARRIERS CONTRACEPTIVE

Increasing apprehension on the part of the medical and lay population concerning the observed and potential risk to health that may accompany the newer methods for the control of fertility has resulted in renewed interest and use of barrier types of contraception by men and women.

Among barrier contraceptives, Intruterine Devices (IUDs) are most popular. Different types of uterine devices are available in the market. Copper T which includes short term and long term copper T and long lasting copper T. Hormone releasing IUDs, fluid filled IUDs, copper 7 etc. Among all IUDs, presently RU 486, the antiprogestin, mifepristone in combination with prostaglandins analogues is popular and highly effective in inducing early abortion (Swahn and Bygdeman, 1990). But RU 486, induces irregularities in menstrual cycle of users and hence not feasible for users (Swahn et al., 1994). Moreover, IUDs are inducing contractions of uterus, uterine bleeding, pelvic infection, uterine perforation, cervical perforation, fundal perforation, etc. Thus, users of IUDs are not fully comfortable with this contraception.

ORAL CONTRACEPTIVE

Oral contraceptive is becoming more and more popular with different types of combinations, with or without steroids, which includes a RH antagonist. GnRH antagonist induce high incidence of menstrual disturbance particularly amenorrhea and oligomenorrhea (Van Look, 1988). LHRH antagonist is also used as a fertility
regulating agent. Its side effects like vaginal dryness, decreased libido and headache are reported in women (Fraser and Baird, 1987). Of greater concern is that prolonged use of LHRH agonists may be associated with hypoestrogenic demineralisation of bone (Fraser, 1988).

VACCINE

At present several researchers are trying to develop a safe and effective method of immunocontraception based on zona pellucida 3 (ZP3) glycoprotein. But ZP3 shows a dramatic loss of primodial follicles in the first month after vaccination. The side effects are poorly understood (Duin et al., 1994).

In recent decades, a search for the "perfect contraceptive" has led to renewed interest in methods of female sterilization for fertility regulation. Despite increased scientific research and public interest however, the ideal sterilization procedure has remained elusive since many of them do not meet the components of such a technique with the requirement which include high rate of effectiveness, low rate of complications, which is reversible, economically affordable, non toxic, with long shelf life etc.

PLANT PRODUCTS AS CONTRACEPTIVE AGENTS

Attempts to develop antifertility drugs from plants have also been made. The problem underlying the research for natural antifertility drugs basically concerns deciding which of the approximately 750,000 species of higher plants should be examined for their potential antifertility and abortifacient activity.
In India, about 2000 years ago important contraceptive agents were the Kandamba fruit, the seed of the red lotus, the palasa seed flower, the salmoli flower, the palm leaf, and the old molasses, etc., which were taken orally (Dutta, 1977). Castor beans were eaten by women to prevent pregnancy. South African tribes use Accacia twigs, Lemon juice and a concoction of mahogany bark was used in Martinique. They all act as spermicides due to their acidic nature.

Extensive studies have been carried out to investigate the anti-fertility effects of different plants, many of which have been screened for their antifertility effects in females (Dela Salo and Henshew, 1954; Casey, 1960; Saxena, 1963; Chaudhary, 1966, 1980; Garg and Garg, 1971; Malhi and Trivedi, 1972; Farnsworth et al., 1975; Setty et al., 1977; Garg et al., 1978; Guerra and Andrade, 1978; Dhawan et al., 1980; Lantum, 1980; Chaudhary and Haq, 1980; Oswiecimoska et al., 1980; Das, 1980; Atal, 1981; Karkhov and Mats, 1981; Popil, 1981; Joshi et al., 1981; Kamboj and Dhawan, 1982; Satyavati, 1984; Aswal et al., 1984; Chinoy and Geetha Ranga, 1983).

Major emphasis by WHO Task Force was placed on identifying agents for use by women that would be taken orally to interfere with the process of implantation or early pregnancy (WHO, Annual Report, 1982, 1983 and 1984). A summarised current status of the studies carried out on assigned and adhoc plants in several Centres were listed (WHO, Annual reports 1983, 1984). The Central Drug Research Institute, Lucknow, in India has also screened numerous plants and their products for their potentiality for use as antifertility agents in both male and female. A brief review of plant and plant products used for female contraception
is presented here.

More than 1800 articles present information pertinent to fertility regulating plants.

Various types of isoflavones, coumestans, Stilbenes alkaloids and other natural products of known structure have been shown to elicit weak estrogenic effects and for a number of reasons are of little significance to human fertility regulation (Farnsworth et al., 1975; Bingel and Farnsworth, 1980). Pakrashi et al. (1975) reported that the stem and bark of Achyranthes aspera Linn, roots of Abroma augusta and flowers of Sesbania aegyptica manifested significant abortifacient effects in female mice. Moreover, antiimplantation effect has also been shown with the petroleum ether extract of Abroma augusta. A significant antifertility effect was observed in rat by using rhizome and leaves of Ananas comosus (Bhaduri et al., 1968) and unripe fruit of Aloe barbadensis (Prakash and Mathur, 1976). More than a dozen plant derived substances of known structure are alleged to have anti-implantation/abortifacient activity in animal trials (Farnsworth et al., 1976; Bingel and Farnsworth, 1980).

The flowers of Hibiscus rosa sinensis Linn (Malvaceae), the common garden plant of India are considered to emollient, were contraceptive properties in female rats (Batta and Shantakumari, 1971; Kholkute et al., 1972; Kholkute and Udupa, 1974).

m-xylohydroguinone, isolated from Pisum sativum L has been widely studied on animals as well as human beings as a contraceptive agent. However, some of the studies in women showed 60% effectiveness of the drug (Sanyal,
A sesquiterpene extracted from the roots of *Aristolochia indica* possessed anti-implantation activity in adult female mice. It also showed antiestrogenic potency in immature female mice when administered along with estrogen (Pakrashi and Saha, 1977).

*Daturalactone* (DQ1) isolated from *Datura quercifolia* and its chemically related compounds (DQ, DQ1, DQ2+1 and DQ, EP) were evaluated for antifertility effects in female albino rats (Chandhoke, 1978). Among all varieties, DQ1 was the most effective antifertility agent but possessed no antiestrogenic activity. Lee et al. (1986) has also reported that the methanol extract of *Datura albus* (root-bark) administered orally, decreased the fertility rate in rats.

Methanol extract of whole plant of *Sida carpinfolia* Linn and chloroform extract of leaves of *Podocarpus brevifolius* altered normal estrous cycle in rats and prevented pregnancy (Kholkute et al., 1978), indicating the presence of steroids in the plant. The petroleum ether and methanol extracts of *P. brevifolius* however, did not manifest any significant antifertility effects.

The aqueous extract of dry berries of *Embelia ribes* was reported to impair the fertility of female mice and rats (Munshi and Rao, 1972; Munshi, 1974). Radhakrishnan et al. (1977), Prakash and Mathur (1976), Rathinam et al. (1976) and Prakash (1979) have also reported significant antifertility effect of the dried berries and seeds of *Embelia ribes* in female rats. Krishnaswamy and Purshothaman (1981) have found that embelin (an active component of *Embelia ribes* is neither estrogenic nor antiestrogenic, while Prakash (1981) has confirmed
that the anti-implantation activity of embelin was due to its potent antiestrogenic action. A dose dependent antifertility effect of *Embelia ribes* has been reported by Bhargava et al. (1984) who observed 69% and 81% effect respectively at doses of 20 and 50 mg/Kg body weight in female rats.

The dried fruits of *Piper longum* (Piperaceae) and its various extracts were screened for antifertility effect in fertile female rats (Kholkute et al., 1978, 1979). The roots of this plant also induced antifertility activity (Garg, 1981).

Byakangelicin, isolated from the fruits of *Ferula alliacea* possessed antigonadotrophic activity against human chorionic gonadotrophin (Pakrashi, 1967). Its another species, *Ferula jaeschkeana* Vatke which is known for various therapeutic uses (Nadkarni and Nadkarni, 1954) also possessed antifertility activity. Prakash (1984a) had reported for the first time that its ethanol extract possessed significant antifertility activity in rats and later Singh et al. (1985) and Prakash (1985b) found that its ethanolic hexane, benzene and chloroform soluble fractions prevented pregnancy in adult female rats.

Antifertility activity of aqueous and alcoholic extracts of *Ligopodium hexosum* (Gaitonde and Mahajan, 1980) and antigonadotropic activity of *Lycopus* species was studied by Findley and Jacobs (1980) and Winterhoff et al. (1980).

Recently, two uterotonic compounds of novel structure *Zoaplantanol* and *Montanol*, have been isolated from the "Zoapatle" plant *Montanoa tomentosa* (Compositae) (Levine et al., 1979). This plant extract has been used as an infusion for centuries in Mexico as an abortifacient (Jiu, 1966; Levine et al., 1979). Oral administration of an aqueous extract is associated with uterine contractions in
pregnant as well as non pregnant women (Gallegos and Cories-Gallegos, 1974).

Extracts of Zoapatle have been administered in women in early pregnancy under controlled conditions (Landgren et al., 1979).

*Mentha orvensis* (Labiatae) was a folk remedy used to terminate pregnancy whose aqueous extract showed uterotonic activity (Kanjanapothi and Taesotikul, 1978; Kanjanapothi et al., 1980).

Abortifacient substances distinct from prostaglandins have been found in various plants, yeast extract and some marine organisms. The abortifacient activity of an extract could be ascribed to its estrogenicity. The activity of pine needle (*Pinus sylvestris*) extract and subterranean cloves (*Trifolium subterraneum*) have been indicated in causing fetal resorption in animals (Biley and Kitts, 1964). A yeast product malucidin was reported to induce fetal resorption in dogs, but the activity could not be generalised to other species and no active constituent was ever isolated (Whitney, 1962; Levi et al., 1969).

Numerous other plants have been studied for their effect on reproduction and fertility in animals. Some possess abortifacient properties viz. alcoholic extract of carrot seeds (Jacob and Morris, 1969; Sharma et al., 1976; Garg and Garg, 1971; Jacob and Kaul, 1973).

The possible contraceptive or abortifacient value of at least some herbs and other substances used by preindustrial people is attested by the World Health Organization (WHO) Task Force on Indigenous Plants for Fertility Regulation (Shain and Lane, 1980). WHO had pursued other projects in the same area specifically isolating and characterising the active agent in substances where
preliminary pharmacological data was available (WHO, 9th Annual Report, 1980). Plants used either as contraceptive agents or abortifacients in Mexico, Paraguay, Hong Kong, Bangladesh and India are currently being studied. Some preliminary results appear quite promising, for example, the Central Drug Research Institute of Lucknow, India has successfully screened extracts from plants used as antifertility agents in India. Fourteen of the more than 100 plants demonstrated greater than 60% antifertility in rats, eight of these were also active in hamsters (WHO, 1977).

Considerable interest had been aroused around the world on Gossypol which was used for fertility regulation in men. A large number of derivatives of gossypol include imino compounds, esters, acetate, formate, metallic complexes. Among them, gossypol acetic acid was the most potent one for its antifertility activity (Wang and Lei, 1979).

Gossypol acetic acid and gossypol formic acid have been tested in a number of laboratory animals and have revealed marked species differences. Rats, hamsters, dog, monkeys showed varying degree of sensitivity, while mice, guinea pig, rabbits, pig, goat, sheep etc, seemed to be resistant (Chang et al., 1980). There appears to be strain differences in the antifertility action of gossypol in rats (Zatuchri and Osborn, 1980; Prasad and Diczfalasy, 1982). A few questions have been raised about its safety and reversibility of antifertility effects. The most serious effect is hypokalemic paralysis reported in some cases and hence gossypol is not safe.

Chinese workers have isolated two diterpenoid ortho esters, Yuanhuacine and Yuanhuadine from the roots of Daphe genkwa, which manifested abortifacient
effects in monkeys (Li-Zhong-Min et al., 1981). It was found to be relatively safe for clinical studies.

Two saponins with antifertility effects from *Gleditschia horrida* have been isolated. They exhibited antifertility effects in female mice. One of the saponin is characterised as triterpenoid attached to more than one sugar (Chou et al., 1971).

Chinoy and Geetha Ranga (1983) and Chinoy and Trivedi (1980) from our laboratory showed that aqueous and alcoholic extracts of *Vinca rosea* leaf possessed anti-implantation and antifertility effects in female and male rats.

Shanti bori a traditional contraceptive pill comprising of *Accacia catchu*, *Accacia arabica* and *Tragia involucerta* was found to inhibit fertility of female rats to about 88%. The pill was found to contain a steroidal glycoside, a triterpene and an alkaloid (Chawdhury, 1984).

Crude extract of *Phaseolus vulgaris* at a dose of 16.7 mg/Kg body weight exhibited antifertility activity including anti-implantation and pregnancy termination activities in mice (Sun et al., 1983)

PAPAYA SEED AS A CONTRACEPTIVE AGENT

*Carica papaya* (Family Caricaceae) is a tropical tree cultivated throughout South America, West Indies, India and in most of the other tropical countries. The fruit is extremely variable in form, size and shape. The skin is smooth and relatively thin, deep yellow to orange when ripe. The central cavity of which is lined with a dryish, pulpy membrane to which adhere numerous black, round and peppery seeds, with a glistening transparent gelatinous coating.
The cold aqueous extract, hot infusion and the resin fractions of the seed stimulate rat intestine. The resin was most potent amongst all the fractions (Bose et al., 1961). The alkaloid solution showed depressant action on blood pressure and intestine. All the fractions paralysed the earth worm (*Pheretima posthuma*) and rat tape worm *in vitro*.

The petroleum ether extracts of the pulp of *Carica papaya* exerted significant antifertility activity in female albino rats (Garg and Garg, 1971). The seeds also decreased the fertility of albino rats (Sareen et al., 1961).

The seeds of the papaya tree contain a glucoside, caricin, which resembles sinigrin, also the enzyme myrosin. The leaf contains another glucoside, carposide and many other glucosinolates (Hanley et al., 1975). The seed of *Carica papaya* yielded a substance with MP 165°C and molecular formula $\text{C}_6\text{H}_{10}\text{N}_2\text{S}$ which was named as carpasemine. The chemical properties of this compound together with its degradation products have been studied and some new derivatives have been produced from it. The identity was also confirmed by mixed melting points of their derivatives (Panse and Paranjpe, 1943). Out of the various compounds isolated from the seeds of the plant and tested against *Ascaris lumbricoides*, only benzylthiourea did not cause any toxic symptoms except local irritation (Dar et al., 1965).

Nineteen different carotenoids were identified in the fruits, the major being cryptoxanthine (48%). Oxycarotenoids were higher in proportion as compared to carotene hydrocarbons. The percentage of cryptoflavin and $\beta$-carotene were 13 and 29.5 respectively. Oxygenated carbonoids present were either hydroxy or epoxy.
carotenoids of β-carotene (Subbarayam and Cama, 1964). The fresh fruit yields 0.001% Carica xanthum \( C_{40}H_{50}O_2 \) a colourless substance of M.P. 16°C and 0.0004% of violaxanthine, \( C_4H_{56}O_4 \), a yellowish red substance of M.P 184°C.

Das (1980) reported that oral administration of papaya seed powder at a dose of 20 mg/day for 8 weeks to male rats inhibited their fertility to about 40%. The histology of testis and other accessory reproductive organs as well as the weight of adrenals, pattern of sperm motility etc., remained unchanged.

In our laboratory, seeds of 4 different varieties of papaya have already been tested, viz. Honey Dew, Ceylon, Ranchi Dwarf and Washington. Among these four varieties, Honey Dew was found to be the most effective as an antifertility agent in both males and females. Therefore in the current work the seeds of Honey Dew variety have been utilised.

The effect of papaya seeds have been tested on male rats, mice as well as guinea pigs using intramuscular subcutaneous and oral routes. All these routes were effective. Various types of extracts were used for the studies at different dosages ranging from 0.1 mg/Kg body weight intramuscularly to 5, 10 and 20 mg/Kg body weight orally. These doses were based on LD_{50} value of papaya seeds (15 gm/kg body weight). According to WHO a LD_{50} value of 5 gm is considered safe. The doses used are also very low in comparison to other studies on plant products.

The 0.5 mg and 5 mg/Kg body weight were effective when given intramuscularly. However, a higher dose of 20 mg/Kg body weight was required to bring about the desired effects when administrered orally.
Studies by Chinoy et al. (1984/85; 1985), Chinoy and Geetha Ranga (1980, 1984), Chinoy and Sam George (1983) and Chinoy et al. (1994c, 1995) have revealed that aqueous extract of papaya seed at a dose of 5 mg/Kg body weight for a period of 60 days by intramuscular and oral routes brought about significant antifertility effects on male guinea pigs and rats. The loss of fertility was attributed to decline in sperm motility, alteration in sperm morphology, and their metabolism, changes in the structure and physiology of epididymis and vas deferens. On the other hand, the treatment did not alter testicular structure and functions as well as the histology of the other reproductive organs. The activity of 3β and 17β hydroxy steroid dehydrogenase, levels of testicular cholesterol, serum cholesterol and FSH and LH levels remained unaltered by the treatment (Chinoy et al., 1984, 1985). All the induced effects were transient and reversible by 2 months of discontinuation of the treatment. Therefore, the results elucidate that extract manifests only post testicular effects in male rodents and that functional sterility could be induced.

The non toxic nature of the aqueous and alcoholic extracts of *Carica papaya* has also been established (Chinoy et al., 1994c, 1995). The extract was non estrogenic in male.

Moreover, Lohiya and his associates have also confirmed our results using different types of extracts, viz. aqueous, ethanol and chloroform from seeds of papaya and reported that they induce sterility in male rats without manifesting any toxic effects. Moreover, the induced effects were totally reversible after withdrawal period (Lohiya and Ravi Bala Goyal, 1992; Lohiya et al., 1992, 1994).
On the whole, comparatively less information is available on effect of papaya seeds in case of females with respect to their precise mechanism of action. However, antifertility activity of papaya seed has been demonstrated in female rats (Garg and Saksena, 1970) and mice (Sareen et al., 1961) mainly due to its anti-implantation activity. According to Farnsworth et al., (1975), the active principle responsible for anti-implantation effect of papaya seed might be 5-hydroxy tryptamine.

In our laboratory, Trivedi and Chinoy (1983) and Chinoy et al., (1994d) reported that Carica papaya seed aqueous and alcoholic extracts manifested temporary anti-implantation effect in female rats with irregularity in cyclicity. The uterine contractile pattern was altered together with internal milieu of uterus of treated rats which resulted in 100% negative fertility rate in these animals. The histological studies have been also shown changes in uterine structure which might not be conducive for implantation (Chinoy and Trivedi, unpublished observation). Thus the extracts manifested anti-implantation and abortifacient effects. All the induced effects of papaya seed extract treatment were completely reversible in case of female rats as in the males upon withdrawal of treatment.

However, there is still paucity of data on females treated with papaya seed extracts. Therefore, in the present study an attempt was made to develop an "ideal contraceptive" which will be easily available, economically affordable in a developing country like India, as well as with reversible and non toxic effects for the females which could be taken orally.