CHAPTER I

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In the present day context, contraceptive biology has a tremendous role to play as population explosion is one of the major problems of developing countries. The history of fertility control could be traced back almost 4000 years, with the discovery of a prescription for contraception written on an ancient Egyptian papyrus. One of the methods suggested therein was the local use of a paste containing ground Acacia (Havemann, 1967).

For any successful contraceptive, various steps in mammalian reproduction such as:

In case of males
a) Spermatogenesis
b) Sperm maturation
c) Transportation of sperm in the female tract

In case of females
a) Oogenesis
b) Ovulation
c) Ovum transport within the fallopian tube
d) Fertilization
e) Implantation
f) Blastocyst development
g) Conceptus maintenance

would be the targets where some kind of alteration is required. All these steps are
regulated by endogenous hormones. Therefore, to bring about a change in the fertility status, the hormonal balance could be altered.

Various methods have been developed and are being devised to control the population especially in the developing countries and a number of contraceptive methods are now available to combat the rapid growth of population. Despite phenomenal advances in fertility control methods, it is clear that every method falls short of possessing all the characteristics of an ideal contraceptive, viz., safe, easily available, cheap, with long self life, and reversible. Different methods fulfill different criteria, and no method is completely devoid of risks, be they serious side effects or failure. As newer methods enjoy varying degrees of popularity, expanded clinical and laboratory data raises newfound concerns. Awareness of the limitations of a method and risks, involved, soon raises apprehensions in a medically conscious patient population and limits its acceptability.

BRIEF REVIEW ON FEMALE CONTRACEPTION

The female reproductive physiology has many steps such as ovulation, ovum transport through fallopian tubes, fertilization, implantation, blastocyst development, and conceptus maintenance, where a slight change of hormonal level or blockage of the fallopian tube besides other causes could lead to infertility.

BARRIER METHODS

A barrier method of contraception is the most logical approach to the interruption of the process of human reproduction. Fertilization is prevented by arresting the progress of sperm.
Barrier methods comprise:

1. **Occlusive pessaries (CAPS)**

   Four types of occlusive pessaries are in current use viz. the diaphragm, cervical cap, vault cap and the vimule.

   **Diaphragm** consists of a thin, latex rubber hemisphere, the rim of which is reinforced by a flexible flat or coiled metal spring. It acts as a physical barrier during sexual intercourse to prevent sperm reaching the cervical mucus. The main disadvantage is it requires premedication, and thereby there is loss of spontaneity with intercourse. It may cause discomfort to the wearer or her partner during intercourse and also cause loss of cervical and some vaginal sensation (Gebbie, 1995).

   **Cervical cap** is shaped like a thimble and is designed to fit closely over the cervix. It is held in place by precise fitting onto the cervix and by suction. It requires accurate selection of cap size and fitting to avoid displacement during intercourse.

   **Vault cap** is made of rubber, is an almost hemispherical bowl with a thinner dome through which the cervix can be palpated. It is designed to fit into the vaginal vault, stays in place by suction.

   **Vimule** is a variation of the vault cap with a thimble-shaped prolongation of the dome. There are three sizes - small (45 mm), medium (48 mm) and large (51 mm). The vimule has fallen into some disrepute because of its association with vaginal abrasions and lacerations, possibly because of the relatively sharp edged rim (Gebbie, 1995).

2. **Female Condom**

   The female condom is a polyurethane sheath, 15 cm long by 7 cm in diameter,
its open end attached to a flexible polyurethane ring. The condom comes in a single size, has a silicone based, non-spermicidal lubricant and is for single use only. Some disadvantages of this condom are: it alters sensation and cause 'rustling' noise during intercourse. It can occasionally be pushed completely into the vagina or penetration can take place outside it and lastly it is very expensive (Gebbie, 1995).

3. **Spermicides**

These contraceptive agents comprise a chemical capable of destroying sperm, incorporated into an inert base. Spermicidal products are available in a variety of different forms viz. creams and jellies, vaginal suppositories, foaming tablets, Aerosal foams and C-film. Usage of spermicides greatly in excess of normal doses can cause lesions and ulceration within the vagina as a dose-related effect (Gebbie, 1995).

**ORAL CONTRACEPTIVES**

The combination of estrogen and progesterone is the most effective type of oral contraceptive formulation. Present-day combined oral contraceptives contain very low doses of hormones. These preparations prevent ovulation and also thicken cervical mucus, making it difficult for sperm to pass through (Hatcher et al., 1997). Common side effects seen are nausea, mild headaches, breast tenderness, slight weight gain and amenorrhea.

Progestogen-only as oral contraceptive was introduced to avoid the side-effects of estrogen and to reduce total exposure to steroids. Progestogens have a multiplicity of actions within the human reproductive system. The important actions are suppression of follicular growth, inhibition of ovulation and suppression of luteal
activity. It modifies cervical mucus and inhibits sperm penetration and prevents implantation with endometrial modifications. It inhibits cyclic release of FSH and LH and hence contributes to suppression of follicular development and ovulation (Fraser, 1995). Disadvantages of this oral contraceptive are that it alters the normal menstrual pattern sometimes causing oligoamenorrhea or even amenorrhea, breast tenderness, and lower abdominal discomfort associated with persisting estradiol secreting follicles.

**Vaginal Rings**

Most steroid hormones are absorbed efficiently through the vaginal epithelium and can be released from a vaginal ring made out of silastic. Rings upto 75 mm in diameter usually stay in the vaginal fornix and fit around the cervix. Combined estrogen - progesterone and Progestogen - only rings are used. These rings are worn for 3 weeks and then removed for 1 week to allow withdrawal bleeding to occur. The ‘3-week-in/1-week-out’ schedule is continued for the life-time of the ring. Like oral contraceptive pills, vaginal rings releasing both estrogen and progestogen are intended to inhibit ovulation with minimal disturbance of vaginal bleeding patterns. Examples of combined rings that are in development include one that releases 3-keto-desogestrel and ethinyloestradiol (organon) and one that releases norethisterone acetate and ethinyloestradiol (Population Council). An example of a progestogen only ring is the levonorgestrel ring with a daily release rate of 20 μg. Also under study is a ring delivering the progestogen ST 1435 (Nestorone) (Van Look, 1995).

**INJECTIONS**

Four injectable steroid formulations viz. Depot-medroxy progesterone acetate
(DMPA), NET-EN (Noristerat), norethindrone enanthate and norethisterone enanthate have undergone extensive clinical trials. DMPA contains a progestin, similar to the natural hormone and is administered in a dosage of 150 mg every three months. It mainly stops ovulation and also thickens cervical mucus, making it difficult for sperm penetration (Hatcher et al., 1997). Common side effects include heavy bleeding, amenorrhea, weight gain, headaches, breast tenderness, nausea, hair loss, less sex drive, and acne in some women. DMPA significantly decreased serum HDL cholesterol concentrations and increases plasma glucose and insulin levels (Ratnam and Prasad, 1983). Norplant Implant system is a set of 6 small, plastic capsules. Each capsule is about the size of a small matchstick. The capsules are placed under the skin of a woman’s upper arm. Norplant capsules contain a progestin. It is released very slowly and supplies a steady, very low dose. A set of Norplant capsules could prevent pregnancy for at least 5 years (Hatcher et al., 1997). But norplant implant induces changes in menstrual bleeding, amenorrhea, headaches, enlargement of ovaries, dizziness, breast tenderness, nausea, weight gain, acne and hair loss.

**INTRA-UTERINE DEVICES (IUD)**

The first IUD, a ring of silkworm gut, was described in 1909. IUDs fall into three categories: inert, copper-bearing and medicated.

**Inert (non-medicated devices)**

Ring devices made of stainless steel are used mainly in China. In the UK, inert IUDs had various ‘open’ designs and were made of silastic, usually with barium sulphate to make them radioopaque (Drife, 1995).
Copper-bearing devices

All IUDs currently available contain copper, which increases contraceptive efficacy and allows the devices to be smaller and easier to fit. Clinical trials have shown that for several devices the lifespan exceeds the recommendations and now all modern copper-bearing IUD's may be left in situ for 5 years (Bromham, 1993).

Flexible devices, the Cu-Fix or flexiguard has six copper sleeves threaded on a prolene filament and a knot at one end of the filament which is pushed into the myometrium with an introducer. This device could be left in place for 5 years and removal is apparently not a problem (Drife, 1995).

Medicated devices

Some IUDs contain a progestogen which is slowly released into the uterus, increasing contraceptive effectiveness and diminishing menstrual blood loss.

A device releasing progesterone (the Progestasert) was marketed in the UK but its use was discontinued as it increased risk of ectopic pregnancy. The levonorgestrel IUD was developed in Finland and consists of a Nova-T frame carrying a silastic sheath impregnated with levonorgestrel, which is released at the rate of 20 μg/day. It can be left in place for up to 5 years. Pregnancy rates as low as 0.2% have been reported, with no increase in the rate of ectopic pregnancy. Fertility returns immediately after the device is removed. This device markedly reduces menstrual flow and dysmenorrhea (Drife, 1995).

All IUDs mentioned above cause a foreign-body reaction in the endometrium, with increased numbers of leucocytes. This reaction is enhanced by copper, which affects endometrial enzymes, glycogen metabolism and estrogen uptake and may
inhibit sperm transport. Endometrial changes produced by the IUD interfere with implantation at an early stage. Steroid releasing IUDs suppress the endometrium in a way similar to the progestogen-only pill, and may inhibit ovulation (Drife, 1995). Disadvantages of IUDs is that for a woman with multiple partners, it increases the risk of pelvic infection. It causes menstrual irregularities, menorrhagia, vaginal discharge and lower abdominal pain.

**IMMUNOLOGICAL ASPECTS OF FERTILITY CONTROL**

The development of immunological means of preventing or disrupting human fertility is one of several approaches to the acquisition of new antifertility methods.

**Immunization against hormones**

In 1936, Parkes and Rowland demonstrated ovulation inhibition in rabbits treated with an antiserum to Ox-pituitary gonadotrophin (Jones, 1982). In female animals, induced immunity to LHRH has been used to study the complex feedback mechanisms controlling LH release. Passive immunization of the ewe causes an abrupt fall in LH secretion. Active immunization of female rats lowers FSH and LH levels and causes ovarian and uterine atrophy (Jones, 1982). Studies have been confined to the rat, in which injection of antibodies to LHRH between days 1 and 7 of pregnancy prevents or delays implantation (Fraser, 1980).

**Immunization against Zona Pellucida Antigens**

Most research on ovum antigens is directed toward the zona pellucida. This acellular, gelatinous layer surrounding the ovum offers perhaps the most promise as
a source of antigens for specific immunological inhibition of ovum viability (Stevens 1983). Passive immunization experiments were initiated to determine the anti-fertility activity of anti-zona antibodies in vivo. Passive immunization of hamsters, mice and rats with antisera raised against ovarian antigens has been found to be extremely effective in blocking fertilization. Immunoglobulin preparations of antisera raised against cumulus-free mouse eggs, or mechanically isolated mouse zonae, inhibit fertilization when given as an intraperitoneal injection. Antibodies raised against pig ovarian antigens induced the formation of a precipitate on the surface of the human zona and inhibited the fertilization of human ova in vitro (Aitken and Richardson, 1980).

3. Immunization against Placental hormone (HCG)

Human chorionic gonadotropin (HCG) is a hormone that is produced by the pre-implantation embryo within a few days of fertilization, and the main function is to maintenance of the corpus luteum, thus ensuring the continued production of progesterone essential for successful implantation. One of the candidate vaccines being studied consists of an apparently unique part of the β-subunit of HCG (β-hCG-CTP) linked to an immunogenic carrier molecule such as tetanus or diphtheria toxoid and injected together with adjuvants that further enhance the immune response (Griffin, 1991).

A Phase I study conducted by WHO with this vaccine preparation showed that it can induce a level of antibodies high enough to neutralize hCG for a period of some 6-12 months. To prolong the antifertility effect, booster injections would need to be given. In India, an anti-hCG vaccine is being tested that consists of the whole
β-subunit coupled to the α-subunit of ovine LH. Studies conducted with this preparation have confirmed its antifertility effect (Van Look, 1995). In addition to lack of long-term safety data, one potentially major problem with anti-hCG vaccines is the individual variability in the antibody response.

EMERGENCY CONTRACEPTION

Emergency contraceptives are methods that could be used to prevent pregnancy following unprotected sexual intercourse. Different methods currently in use are:

1. **High – dose estrogens**

   High doses of estrogens given within 72 – hours of the unprotected intercourse has a very high contraceptive efficacy, the pregnancy rate is 0 to 0.6 % with most of the regimens. Diethylstilbestrol (DES), ethinyl estradiol (EE) and conjugated equine estrogens are used as emergency contraceptive agents (Haspels, 1994; Van Look, 1987). However, the high incidence of side effects viz, adenocarcinoma of the vagina and congenital defects of the genital tract in female offspring during early pregnancy have restricted the use of high dose estrogens for emergency contraception.

2. **Yuzpe Method**

   A Canadian physician Albert Yuzpe showed that a single dose of 100 μg of estrogen and 1.0 mg of dl-norgestrel rendered the endometrium out of phase. Since then the estrogen-progestin combination, also commonly referred to as Yuzpe method has been used. Different combinations of estrogen and progestins have been tried. The most typical formulation contains 200μg of ethinyl estradiol and 2.0 mg of dl –
norgestrel which is given in two divided doses. The treatment is initiated within 72 hours of the unprotected sex and the second dose is repeated 12 hours later. The failure rate of the Yuzpe method ranges from 0.2% to 2%. The side effects are nausea, vomiting, breast tenderness, abdominal pain, dizziness and headache (Yuzpe, 1985; Sanchez-Borrego and Balasch, 1996).

3. Levonorgestrel

Levonorgestrel is used as a post-coital contraceptive. A study was conducted among 50 fertile women in Hungary to test the efficacy of 0.75 mg of levonorgestrel administered immediately after intercourse, which occurred around the time of ovulation. There were no pregnancies in 150 cycles studied when levonorgestrel was given during the early follicular phase. Administration around ovulation caused variable effects. Ovulation might be blocked or follicular activity may be followed by deficient luteal function, or the women may ovulate normally. Research is still on to determine how levonorgestrel acts when it is administered after ovulation has occurred (Von Hertzen and Van Look, 1996).

4. Copper-IUD’s

Cu-IUD as a method for emergency contraception was introduced in the late 1970s by Lippes and co-workers. The IUDs, prevent implantation which is attributed to endometrial changes resulting from the presence of the device and the copper ions, and possibly a direct embryotoxic action of copper (Lippes et al., 1976).

The emergency use of a Cu-IUD is a highly effective method to prevent pregnancy, the failure rate is probably not higher than 0.1 per cent. Cu-IUD could be
inserted upto 5 days after intercourse or upto 48-72 hours later than hormonal methods. The longer time span is due to the ability of an IUD to prevent implantation. Postcoital IUD insertion is particularly useful when the hormonal methods are no longer effective. Some of the disadvantages of the IUD are: (1) it may be difficult to insert in young and nulliparous women; (2) some abdominal discomfort may occur; (3) risk of infection, and (4) in some cases relatively long-term contraceptive protection may not be desired (Van Santen, 1995).

POTENTIAL FUTURE METHODS FOR EMERGENCY CONTRACEPTION

The currently available above mentioned methods do not meet all the requirements of an ideal emergency contraceptive. There is an urgent need for new and better methods of emergency contraception. The hormonal methods which are being investigated include an antiprogestin mifepristone and a progestogen danazol.

1. **Antiprogestogens**

Progesterone is indispensable for the establishment and maintenance of pregnancy. Any substance interfering with the synthesis, secretion or peripheral actions of progesterone would have antifertility effects (Puri, 1995). Mifepristone (RU-486) is a potent progesterone antagonist which blocks the action of progesterone both at the endometrial and pituitary levels. Mifepristone disrupts follicular development and arrests ovulation. It impairs endometrial development and prevents implantation (Puri and Van Look, 1991; Gemzell-Danielsson et al., 1993). To assess the efficacy and side effects of lower doses of mifepristone in emergency contraception, WHO has carried out a multi centre study. According to interim results, all doses (10, 50 and 600 mg)
appeared to be equally effective and prevented about 90 per cent of the pregnancies. These studies need to be extended to determine whether mifepristone could be used in one cycle without causing disturbances in the menstrual cycle rhythms (Von Hertzen, 1996).

2. Danazol

Danazol is a progestogen with antigonadotrophic activities. It also prevents implantation by rendering the endometrium out of phase. The use of danazol for emergency contraception has been found to be more effective as compared to the Yuzpe regimen (Zuliani et al., 1990). Among the 990 women who were treated with 800 mg danazol (two doses of 400 mg at 12 hour intervals) within 72 hours of the unprotected intercourse, only 17 pregnancies occurred (pregnancy rate: 1.7%). Increase in the dose of danazol further reduced the pregnancy rate. A more recent study, however, failed to confirm these findings (Webb et al., 1992). The pregnancy rate in the danazol treated group (600 mg given twice, 12 hour apart) was 4.7 per cent, which was nearly the same as the number expected with no treatment. This study questions the usefulness of danazol in emergency contraception. Danazol is contraindicated in women with undiagnosed or abnormal bleeding, impaired liver, kidney or heart function, or porphyria, epilepsy or migraine (Webb et al., 1992).

In recent decades, a search for the “perfect contraceptive” has led to renewed interest in methods of female fertility regulation. Despite increased scientific research and public interest however, the ideal contraceptive procedure has remained elusive since many of them do not meet the components of such a technique with the requirement which includes high rate of effectiveness, low rate of complications, is
PLANT PRODUCTS AS CONTRACEPTIVE AGENTS

Plants, the living chemical factories of nature, synthesize many medicinally important drugs. Since time immemorial, human beings have used these drugs to relieve pain and cure many diseases. This last decade has seen a sudden resurgence of interest in natural products. Plants have served as natural sources of antifertility substances. 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 activities.

In India, about 2000 years ago important contraceptive agents were the Kadamba fruit, seeds of the red lotus, palasa flower, Salmoli flower, palm leaf, and the old molasses, etc., which were taken orally (Dutta, 1977).

Many varieties of plant substances or their derivatives have been tested for antifertility effects in laboratory mammals of either sex (Dhawan et al., 1977). But, not much attention has been given to the mode of antifertility action of these substances as well as their effects on the biochemical makeup of the mammalian genital organs.

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 summarized 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 males and females. More than 1800 articles present information pertinent to fertility regulating plants. The plant products used for female contraception are presented here.

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, anti-implantation effect has also been shown with the petroleum ether extract of Abroma augusta. A significant antifertility effect was observed in the rat by using the rhizomes and leaves of Ananas comosus (Bhaduri et al., 1968) and unripe fruit of Aloe barbadensis (Prakash and Mathur, 1976).

The flowers of Hibiscus rosa sinensis Linn (Malvaceae), the common garden plant of India were considered to have contraceptive properties in female rats (Batta and Santhakumari, 1971, Kholkute et al., 1976; Kholkute and Udupa, 1976).

M - xylohydroquinone, isolated from Pisum sativum L. has been widely studied as a contraceptive agent on animals as well as human beings. However, some of the studies in women showed 60 % effectiveness of the drug (Sanyal, 1956).

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 carpinifolia* Linn and Chloroform extract of leaves of *Podocarpus brevifolius* altered normal estrous cycle in rats and prevented pregnancy (Kholkute et al., 1978a), indicating the presence of steroids in the plants. 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 (Kholkute et al., 1978b). Krishnaswamy and Purshothaman (1981) had 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.

The dried fruits of *Piper longum* (Piperaceae) and its various extracts were screened for antifertility effect in fertile female rats (Kholkute et al., 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 its various therapeutic uses (Nadkarni and Nadkarni, 1954), also possessed antifertility activity. Prakash (1985a) 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 *Lygodium fleuosum* (Gaitonde and Mahajan, 1980) and antigonadotropic activity of *Lycopus* species was studied by Findley and Jacobs (1980) and Winterhoff et al. (1980).

Two uterotonic compounds of novel structure, Zoapatanol and Montanol have been isolated from the “Zoapatle” plant *Montanoa tomentosa* (Compositae) (Levine, 1979). This plant extract has been used as an infusion for centuries in Mexico and as an abortifacient (Jiu, 1966; Levine, 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).

*Mentha arvensis* (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 (Biely 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 effects on reproduction and fertility in animals. Some possess abortifacient properties viz. Alcoholic extract of carrot seeds (Sharma et al., 1976; Garg and Garg, 1970a; Garg, 1975).
Considerable interest had been aroused around the world on Gossypol which was used for fertility regulation in men. Gossypol acetic acid was the most potent derivative found among others 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, monkey showed varying degree of sensitivity, while mice, guinea pig, rabbits, pig, goat etc., seemed to be resistant (Chang et al., 1980). 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 (Prasad and Diczfalusy, 1982).

Two saponins with antifertility effects from Gleditschia horrida have been isolated. They exhibited 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) showed that aqueous and alcoholic extracts of Catharanthus roseus (Vinca rosea) leaf possessed anti-implantation and antifertility effects in female and male rats.

Shanti bori a traditional contraceptive pill comprising of Acacia catechu, Acacia 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, 1980).

Indigenously available neem oil in its natural form was tested for its spermicidal activity (in vivo and in vitro). Undiluted neem oil was found to possess strong spermicidal action (within 30 sec) against rhesus monkey and human spermatozoa in vitro (Sinha and Riar, 1984). When used intravaginally, in a dose of
20 μl in rats, and 10 ml in rhesus monkeys and human subjects before sexual intercourse, the oil was found to be 100% effective in preventing pregnancy in the test subjects. The oil did not reveal any side effects, as confirmed by histopathological studies (Sinha and Riar, 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).

The crude extract of *Balanites roxburghii* at a dose of 500 mg / kg for 45 days exhibited antifertility activity in male mice (Rao and Shah, 1997). The induced effects were reversible on withdrawal of treatment. The antifertility activity of *Phyllanthus amarus* extract in male mice was studied. The extract did not reveal any side effects (Rao et al., 1997).

The possible contraceptive or abortifacient value of at least some herbs and other substances used by preindustrial people is attested by the 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).
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 seeds of the papaya tree contain glucoside, caricin, which resembles sinigrin, also the enzyme myrosin. The leaf contains another glucoside, carposide and many other glucosinolates (Hanley et al., 1983). The seed of Carica papaya yielded a substance with MP 165 °C and molecular formula C₆H₁₀N₂S 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).

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 β - carotene were 13 and 29.5 respectively. Oxygenated carbonoids present were either hydroxy or epoxy carotenoids of β - carotene (Subbarayan and Cama, 1964). The fresh fruits yield 0.001 % Carca xanthum, C₄₀H₅₀O₂, a colourless substance of M.P. 16 °C and 0.0004 % of violaxanthine, C₄H₅₄O₄, a yellowish red substance of M.P. 184 °C.

The cold aqueous extract, hot infusion and the resin fractions of the seed
stimulate rat intestine. The resin was most potent amongst all fractions (Bose et al., 1961). The alkaloid solution showed depressant action on blood pressure and intestine. All the fractions paralysed the earthworm (*Pheretima posthuma*) and rat tapeworm *in vitro*. These compounds act as vermifuge.

The petroleum ether extracts of the pulp of *Carica papaya* exerted significant antifertility activity in female albino rats (Garg and Garg, 1970b). The seeds also decreased the fertility of male albino rats (Sareen et al., 1961).

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.

Saha et al. (1961) showed that papaya fruit latex possesses oxytocic, emmenagogue and/or abortifacient properties.

Kapoor et al. (1974) tested three different extracts of papaya seeds viz., petroleum ether, alcoholic and aqueous for their possible anti-ovulatory activity in rabbits. The petroleum ether extract of papaya seed inhibited ovulation in 20% of rabbits but the alcohol and aqueous extracts lacked anti-ovulatory activity in these animals (Kapoor et al., 1974).

Gopalakrishnan and Rajasekharasetty (1978) showed that the pulp of unripe fruits of papaya had abortifacient activity in pregnant rats. Similarly, Kamboj (1988) has reviewed different Indian medicinal plants with interceptive activity and mentioned that unripe fruit and seed of papaya showed abortifacient and anti-implantation activity in female rodents.
In our laboratory, seeds of 4 different varieties of papaya have already been tested, viz. Honey Dew, Ceylon, Ranchi Dwarf and Washington. Amongst these four varieties, Honey Dew was found to be the most effective as an antifertility agent in male rat (Chinoy et al., 1996). Therefore, in the current work the seeds of *Carica papaya* variety Honey Dew have been utilized.

The effects of papaya seeds have been tested on male and female rats, mice as well as guinea pigs using intramuscular, subcutaneous and oral routes. All these routes were effective in bringing about antifertility effects. Amongst all these routes, intramuscular route brought about antifertility effect after 7 days of treatment. However, oral administration was preferred as it is more feasible and safe. Various types of extracts viz. Aqueous, Alcohol and Benzene 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 extracts viz. 15 gm / kg body weight. According to WHO standards, 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 administered orally.

**Studies in male rodents**

Studies by Chinoy and her group (Chinoy and Sam George, 1983; Chinoy and Geetha Ranga, 1984; Chinoy et al., 1984/85; 1994; 1995a; Chinoy and Padman, 1996) have revealed that aqueous, alcoholic and benzene extracts of papaya seeds at a dose of 5 mg/kg body weight for 60 days by intramuscular route and 20 mg/kg body
weight for 30 days by oral route brought about significant antifertility effects on male mice 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 the structure and functions of the testis as well as the other reproductive organs. The activities of 3β and 17β hydroxy steroid dehydrogenases in testis, levels of testicular cholesterol, serum cholesterol as well as FSH and LH levels remained unaltered by the treatments (Chinoy et al., 1984/85; 1995a; Chinoy and Padman, 1996). All the induced effects were transient and reversible by 2 months of discontinuation of the treatment. Therefore, the results elucidate that the extracts manifested post-testicular effects in male rodents and that functional sterility could be induced. The non-toxic nature of the aqueous, alcoholic and benzene extracts of *Carica papaya* has also been established (Chinoy et al., 1994; 1997b, Padman, 1995). These extracts were also non-estrogenic in nature (Chinoy et al., 1994; Chinoy and Padman, 1996; Joshi, 1995; Padman, 1995).

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).

**Studies in female rodents**

*Carica papaya* seed aqueous, alcoholic and benzene extracts manifested temporary anti-implantation effects in female rats with irregularity in cyclicity (Chinoy...
and Trivedi, 1980; Joshi and Chinoy, 1996; Chinoy et al., 1995b, 1997a). The internal milieu of uterus and its contractile pattern of treated rats were altered which resulted in 100% negative fertility rate in these animals. The histological studies have also shown changes in uterine structure, which might not be conducive for implantation. Thus, the extracts manifested anti-implantation and abortifacient effects. All the induced effects of papaya seed extracts were completely reversible in case of female rats as in the males, upon withdrawal of treatment.

On the whole, comparatively less data is available on the 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 Garg, 1970b) 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. However, it remains to be seen if only this compound or others too are involved in the anti-implantation process.

The fractionation of the aqueous and alcoholic extracts has also been attempted (Chinoy, unpublished data) as well as the Phase-I trials have been initiated in collaboration with other laboratories.

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 a contraceptive with reversible and non-toxic effects for the females which could be taken orally. Therefore in the present study, the Carica papaya seed extracts viz. Methanol, butanol, mixture of aqueous and benzene (1:1) and mixture of aqueous, alcohol and benzene (4:2:1) have been tested in female mice for their
antifertility potential. Studies on reversibility of the treatment, estrogenicity and toxicity if any, were also carried out. The data elucidates that these extracts are promising candidates for female contraception.