CHAPTER I
INTRODUCTION

The growth of human population has assumed alarming proportions and the necessity of ensuring adequate food and clothing, housing and health, and a reasonable standard of life for the great majority of the population has prompted the people and the governments to look for safe and affective ways to control fertility.

Vitamins in general and particularly Vitamins A, B, C and E are necessary for the reproductive process. Lack of adequate vitamin intake in animals suspends estrous activity and causes sterility (Moe et al., 1967). Furthermore, it has been shown by Warkany (1955), and Watteville et al., (1954), that faulty vitamin intake by pregnant mothers may give rise to various developmental alterations and even cause congenital anomalies in the fetus.

Vitamin C is known to be an important biologically active reductant which is widely distributed in animal tissues and is found in high concentrations in reproductive tissues, pancreas, thyroid, liver and adrenal. It occurs in the free and bound form (ascorbigen) in animal tissues and the latter is stored. The storage capacity of each tissue varies greatly (Malakar, 1963; Chinoy, 1971, 1972; Chinoy, 1978 and several other publications from our laboratory). Liver, adrenals,
hepatopancreas, brain, kidney, gonads, accessory sex glands and muscles are major sites for storage. The storage and tissue distribution of ascorbic acid in rats are under the hormonal influence of testosterone in males and estrogens in females (Chinoy and Seethalakshmi, 1978a; Chinoy and Rao, 1979; Chinoy et al., 1979a), and could be altered under stress conditions. A sex difference in the occurrence of the ascorbic acid has also been noted so that the male rats have higher ascorbic acid in their tissue than the females (Stubbs and Mckernan, 1967). The tissues containing higher ascorbic acid concentration possess concomitantly higher tempo of ascorbate turnover.

The localization of ascorbic acid has been demonstrated histochemically using a modified technique in a wide variety or reproductive tissues of male and female rats, mice, guinea pigs and chicks (Chinoy, 1969a, b; Chinoy et al., 1974a, b; Chinoy and Sanjeevan, 1978a).

Giroud (1951), Tonutti and Plate (1937) have revealed that ascorbic acid is located in the theca interna and granulosa of the follicle. Similarly, Chinoy et al. (1979a) reported that in the normal intact rat ovary, the germinal epithelium, and the stromal cells were rich in Vitamin C. The primary follicle cells, the Graafian follicles, the thecal cells and the corpora lutea were also intensely stained. In our studies the nuclei showed more intense ascorbic acid
staining as compared to the cytoplasm. This was a characteristic feature of all the tissues and corroborates with the data of AA-macromolecule charge transfer complex (CTC) formation (Chinoy et al., 1978a). The importance of such a mechanism of energy transfer for biological processes is well known (Szent-Györgyi, 1960). The concentrations of ascorbic acid are higher in the reproductive tissues than in liver, which is known to synthesize AA (Chinoy, 1978, 1980; Chinoy and Seethalakshmi, 1978a; Chinoy et al., 1979a).

Guinea pigs, primates and human being are incapable of synthesizing ascorbic acid and depend on the dietary sources for obtaining it (Sebrell and Harris, 1967), whereas, the rats can synthesize it as an intermediary product of their carbohydrate metabolism. Some authors have contended that embryonic tissue is capable of ascorbic acid synthesis and claim to have shown in the human fetus. However, the reproductive tissue of all these animals have AA metabolizing capacity as they all possess the necessary enzymes. The rats have greater metabolizing capacity as compared to the guinea pigs. Although, the reproductive tissues are known to store large amounts of AA, their ascorbate synthesizing capacity is not known.

A high turnover of ascorbic acid has been associated with tissues having a higher tempo of metabolic activity and also growing and developing tissues (Chinoy et al., 1974a, b;
Chinoy and Parmar, 1975a, b; Chinoy, 1978). Its involvement in the activation of a number of enzymes have been well established (Harrer and King, 1941; Sebrell and Harris, 1967; Kutsky, 1973; Lewin, 1976). The importance of ascorbic acid in biosynthetic reactions involved in growth and regeneration of animal tissues and in cellular metabolism have also been elucidated (Chinoy, 1978).

Chinoy and her associates have carried out work on the metabolic significance of ascorbic acid in the reproductive physiology of a variety of animals including human beings (see review; Chinoy, 1978).

Several oxidoreductive reactions of the body tissues involve the participation of ascorbic acid, whose oxidation is catalyzed by a special peroxidase (Gurevich, 1963; Gorbunova, 1966; Chinoy, 1970, 1973), which monovalently oxidizes ascorbic acid to its free radical, monodehydro-ascorbic acid (MDHA), an unstable compound which has stronger reducing properties than ascorbic acid by virtue of possessing an unpaired electron (Yamazaki, 1977; Chinoy, 1978), and finally, MDHA is converted to dehydroascorbic acid (DHA), whose reduction in animal tissues is brought about by glutathione (Sebrell and Harris, 1967).

Extensive studies from our laboratory have revealed for the first time, that MDHA functions as a source of electron energy for several oxidoreductive reactions in animal tissues
Electron spin resonance (ESR) spectrophotometry revealed that ascorbic acid yields a characteristic ESR spectrum, which is a doublet with a g value of 2.004 and a line width of 1.8 gauss, and was recorded for the first time in cauda epididymal sperm suspension of rats and normospermic human semen in our laboratory (Chinoy and Bueh, 1977; Chinoy et al., 1978b, 1979b).

The mechanism of action of AA involves formation of MDHA and charge transfer complexes (CTC) with macromolecules, viz., proteins, nucleic acids, and steroids (Slifkin, 1971; Swartz et al., 1972; Chinoy et al., 1978a). The steroid ascorbate CTC formation was reported for the first time by Chinoy et al. (1978a). Upon breaking of the complex, the free radicals of both the interacting substances would be formed and MDHA is made available once again. Ascorbic acid inhibits the activity of phosphodiesterases (Lewin, 1976) and thus would increase the levels of cyclic-AMP, which is a second messenger involved in activation of many enzymes. Similarly, ascorbic acid activates numerous enzymes (Kutsky, 1973; Sebrell and Harris, 1967; Chinoy and Seethalakshmi, 1978b; Chinoy, 1978).

It has also been demonstrated from our laboratory that the tissue distribution and concentration of ascorbic acid in steroidogenic tissue and ascorbic acid synthesizing tissue in normal, gonadectomized and sex hormone treated gonadectomized
male and female rats are inter-related. Further they also found that the concentration, metabolism and synthesis of ascorbic acid in liver and adrenal of male and female rats were observed to be more androgen-sensitive than estrogen sensitive (Chinoy and Rao, 1979).

An inter-relationship exists between the metabolisms of ascorbic acid and testosterone, so that the synthesis of AA is under the control of gonadal hormones in cockerels and rats (Dieter, 1969; Majumder and Chatterjee, 1974). On the other hand, the involvement of ascorbic acid in steroidogenesis in gonads and adrenals has also been demonstrated (Agrawal and Laloraya, 1977; Datta and Sanyal, 1978; Chinoy et al., 1980a). It has been postulated that trophic hormones, i.e. LH and FSH, c-AMP and AA play a combined role in the regulation of adrenal and gonadal steroidogenesis acting at different levels of hypothalamo-hypophyseal-adrenal-gonadal axis. Moreover all these substances were potent inducers of steroidogenesis by stimulating membrane bound adenyI cyclase (Datta and Sanyal, 1978).

Recently, Chinoy et al. (1980a, b) and Chinoy and Asok Kumar (1980) have observed a depletion of testicular ascorbate content, with a sudden spurt in its metabolism concomitant with increase in serum testosterone levels with the onset of puberty and with passing of the first wave of spermatozoa through the epididymis in rats. This increased ascorbic acid
concentration is utilized by spermatozoa since a high ascorbic acid turnover has been correlated with greater sperm motility and metabolism (Chinoy and Buch, 1977). Thus an inter-relationship exists between the metabolism of ascorbic acid and testosterone.

Ascorbic acid synergizes with testosterone and potentiates its anabolic action in androgen target organs in castrated animals as well as those under various treated conditions. Thus it helps in maintaining the structural and functional integrity of the androgen target organs (Chinoy, 1978; Seethalakshmi and Chinoy, 1978; Buch et al., 1978; Chinoy et al., 1978b,c, 1979b; Chinoy M.R. and Chinoy N.J., 1979).

The ovarian ascorbic acid depletion is influenced by LH (Parlow, 1958, 1961). Similarly, a dose dependent AA and cholesterol depletion in Leydig cells by LH was demonstrated in immature rat testis (Chinoy et al., 1980b). PGE₁, E₂ or F₂ₐ also produced a highly significant decrease in the ovarian content of ascorbic acid in intact and hypophysectomized immature rats with luteinized ovaries due to previous treatment with pregnant mare serum gonadotrophin (PMSG) and human chorionic gonadotrophin (hCG) (Sato et al., 1974).

Agrawal and Laloraya (1979) observed that ovarian ascorbate is at its lowest concentration at estrus and
reaches its peak at diestrus. LH has been shown to induce peroxidase in the corpora lutea accompanying the depletion of ascorbate in the rat ovary, and a suggestion has been made that the free radical of ascorbate produced by the action of peroxidase on ascorbic acid, may trigger oxidation of pregnenolone through a free radical mechanism, thus bringing about rapid formation of progesterone (Agrawal and Laloraya, 1977).

Guraya (1974), on the basis of presence of sudanophilic granules and its changes under gonadotrophic hormone action, has suggested the possibility of interstitial gland cells acting as sites for steroidogenesis. These sites are well known to be associated with storage of lipid material including phospholipids and triglycerides. The observation that cholesterol could be demonstrated in interstitial gland cells and under conditions of lowered progesterone secretion, but not during active secretion, might suggest that the mobilization of the precursors for the biosynthesis of progesterone is controlled by the corpus luteum. Since no activity of peroxidase could be demonstrated in interstitial gland cells, it appears to be an unlikely site for luteal steroidogenesis (Agrawal and Laloraya, 1978a).

The corpora lutea of many mammals are chief sites of conversion of $^{14}$C-acetate to progesterone (Zander et al., 1959; Hammerstein et al., 1964; Savard et al., 1965). The enzyme activities such as those of $\Delta^5$-3$\beta$-OH-steroid
Dehydrogenases and 17-β-HSD in the mammals have been correlated with the synthesis of 2\(\alpha\)-OH steroid and progesterone in cells of the theca interna, interstitial gland cells and corpus luteum (Levy et al., 1959; Taylor, 1961; Rubin et al., 1963, 1965; Wiest and Kidwell, 1965). The secretory sites of progesterone in the normal and pregnant rats are the corpora lutea and placenta, respectively. However, since the peroxidase-mediated reaction are manifold faster than dehydrogenase reactions, the association of high peroxidase activity in these regions and lack of activity in growing follicles as well as in interstitial gland tissues suggest that peroxidase may be involved in the luteal steroidogenesis (Agrawal and Laloraya, 1977).

Ascorbic acid plays a role of extraordinary importance in pregnancy (Botella, 1940; Botella and Hernandez-Arana, 1942), as shown by Jose Botella-Llusia (1973) and confirmed by a number of investigators (Giroud and Boisselot, 1951; Neuweiler, 1943; Tonutti and Plate, 1937), the placenta stores ascorbic acid. During pregnancy, ascorbic acid progressively accumulates, reaching a maximum at term. Blood levels and urinary excretion values experience a similar progressive rise during pregnancy. Not infrequently, placental storing may drain this vitamin from the maternal body and a relative maternal deficiency may coexist along with an abundance of the vitamin in the placenta and in the fetal body.
Fetal storage of ascorbic acid, particularly by the adrenal cortex and the liver, acquires considerable importance near term. Vitamin C was shown by Neuweiler (1943) to be present in high concentrations in the lactating breast, which suggests that it is essential for, or at least plays an important role in the secretion of milk.

Ascorbic acid avitaminosis in experimental animals causes lack of corpus luteum formation and symptoms of adrenal insufficiency. In addition to serious disturbances of adrenocortical origin, clinical scurvy equally leads to suspension of menstruation, as well as to sterility or frequent abortion.

Vitamin C is currently held to be directly implicated in the synthesis of both corticoids and progesteroids. Vitamin C is stored specifically in those organs which like the adrenal, produce corticoids or, like the corpus luteum, the placenta and the adrenal cortex produce progesterone.

Vitamin C deposits in the fetal adrenal are not apparent before the seventh month of pregnancy. That is not until the gland has started secreting corticoids. Both androgen and estrogen syntheses by the adrenal cortex during the first months of gestation, when there are still no ascorbic acid deposits, have been demonstrated to occur.

Animals maintained on Vitamin C deficient diets for more than one month failed to survive adrenalectomy. Obviously, despite the presence of corpora lutea, rats with experimentally induced scurvy lost their capacity to
withstand bilateral adrenalectomy. Injections of LH or progesterone into control animals failed to produce any signs of improvement, which seems to indicate that, in the absence of ascorbic acid, progesterone cannot be converted to corticoids and thus cannot prevent death in adrenoprival animals. Another group of control animals treated with ascorbic acid were able to survive the effect of adrenalectomy even without progesterone administration.

The significance of these findings seems to deserve some further comment concerning the existence of a fundamental difference between the human species and certain animal species with regard to their respective capacity for endogenous Vitamin C synthesis. As vitamin C is not synthesized within the human body, we depend entirely on dietary supply in order to maintain the necessary requirement for this biocatalyst. In contrast, the reproductive capacity of the rat, which is capable of synthesizing this substance is hardly affected by dietary vitamin C intake. However, if this source of vitamin C production in the rat (the adrenal) is removed, the biocatalyzing action by vitamin C is completely lost. Even though the exact biochemical mechanism involved is not known, it seems plausible to assume that vitamin C acts as a biocatalyzer of C-21 steroid synthesis and that it therefore intervenes in a decisive manner in progesterone and corticoid biogenesis.
Ascorbic acid is an anti-stress factor as it helps in the detoxification of the stress-induced histamine via its increased synthesis and utilization and thus has beneficial effects on the tissue metabolism during and following drug administrations (Zannoni et al., 1972; Subramanian et al., 1974; Nandi et al., 1974; Chinoy and Sheth, 1976; 1977a, 1978, 1979; Chinoy and Seethalakshmi, 1977a, b; 1978c, d; Buch et al., 1978). Thus ascorbic acid plays an important role in normal metabolism and also in restoring normalcy after various altered physiological conditions. Aarts (1966) reported that male rats responded with a greater urinary excretion of ascorbic acid following the drug treatment than females.

Ascorbic acid is involved in protein, nucleic acid, carbohydrate, lipid, mineral and muscle metabolism (Chinoy J.J. et al., 1974; Chinoy and Sheth, 1977b; Chinoy and Kshatriya, 1977; Chinoy, 1978). Vitamin C also possesses important inter-relationship with several hormones. Serotonin, epinephrine, norepinephrine, are produced under the influence of AA (Chinoy, 1978). Its interplay with testosterone has been discussed earlier.

The important role of ascorbic acid in human and rat sperm motility and metabolism was reported for the first time from our laboratory (Chinoy and Buch, 1977; Chinoy et al., 1978a, b, c, 1979b; Chinoy and Sanjeevan, 1978b; Rao et al.,
1978). The important implications of ascorbic acid in the prophylactic treatment following vasectomy, cyproterone acetate treatment and other antifertility drugs without interfering with the contraceptive purpose of the treatment have also been demonstrated (Chinoy, 1978; Chinoy et al., 1978b, 1979b).

Based on these evidences, it is suggested that tissue metabolism is energised not only by high energy phosphate (\( \sim \) P), but also via the paramagnetic electron flow from MIHA, the electron donor which plays an important role in the energy supply to the tissues during normal and altered physiological conditions (Chinoy, 1978; Chinoy et al., 1979b).

The uterus changes in size and shape during reproductive life. Four anatomic regions are noted along the length of the uterus: fundus, corpus, isthmus and cervix. The uterine lumen exhibits maximal anteroposterior flattening, and tapers to the isthmus at the junction of the corpus and cervix; then it becomes fusiform in the cervix.

The uterus is made up of three major concentric layers: 1) a thin outer Perimetrium or peritoneal covering; 2) a massive myometrium of smooth muscle, and connective tissue; and 3) an innermost endometrium. From the physiologic standpoint, only two layers are recognized—the myometrium and the endometrium.

The frequency and patterns of myometrial contraction vary with different stages of the menstrual cycle (Csapo and
Changes in contractility may be a reflection of variations in myometrial ultrastructure during the menstrual cycle. Ultrastructural studies of the myometrium have been carried out primarily in laboratory animals. Before estrus, the rat myometrium consists mainly of muscle cells and a few fibrocytes. With the onset of estrus, fibroblasts predominate and myocytes contain an increased number of ribosomes and enlarged Golgi complexes and sarcoplasmic reticulum (Ross and Klebanoff, 1967).

Fluctuating levels of estrogen and progesterone throughout the menstrual cycle cause remarkable changes in the structure and ultrastructure of the uterus, and in the biochemical and biophysical characteristics of endometrial secretions. This cyclic response is prominent in the outer layers of the endometrium (Hafez, 1980).

In the proliferative phase of the cycle, the glands, under the influence of estrogen, become highly coiled. During the secretory phase of the cycle, when progesterone levels are high, the endometrial glands become filled with the endometrial fluid, which is then secreted into the uterine lumen. The endometrial secretion provides an optimal environment for the transport and capacitation of sperm and the nutrition of the preimplantation blastocyst. If the ovum is fertilized, the endometrium continues to
grow and become the decidua of pregnancy. The stromal
cells swell and the endometrial glands become relatively
scarce. In the absence of a fertilized ovum in the uterus,
the corpus luteum and endometrium regress, and menstruation
resumes about 14 days after ovulation. According to an
extensive review by Wynn (1977), there are three major
changes occurring in the surface and gland epithelium
associated with an ovulatory cycle: subnuclear accumulation
of large deposits of glycogen in the gland cells; appearance
of giant mitochondria; and the formation of nucleolar
channel system.

The surface of the endometrium in the secretory phase
appears densely covered by secretory cells with bulging
apices stippled with microvilli, and numerous ciliated cells
concentrated primarily around gland openings. In the luteal
phase, progesterone is acting on the uterus. The surface
ultrastructure varies with region of the uterus, the phase
of the menstrual cycle, onset of implantation, aging,
administration of steroid contraceptives and the presence
of IUDs (Hafez and Ludwig, 1977). Cyclic changes have been
observed in shape, distribution and number of apical
microvilli, ciliation, and secretory activity.

Ludwig and Metzger (1976) suggest that during progestogen
treatment, areas of exfoliation occur as a result of radial
ruptures, which produce deep clefts and intercellular spaces,
separating large groups of cells. Loosening and extrusion of cells then take place. Prostaglandins especially PGF$_{2a}$ appear to play a role in the initiation of uterine bleeding, perhaps by stimulating myometrial contractility and inducing vasoconstriction. Endometrial tissue levels are highest during the menstrual phase, especially in dysmenorrheic women (Singh et al., 1975). Serum levels of prostaglandins do not appear to fluctuate with the cycle (Van Orden et al., 1977).

Most investigators support the concept that endometrial repair involves new formation of superficial epithelium and is initiated when the zona basalis is denuded of its overlying zona spongiosa. However, desquamation has been shown to occur only in the most superficial layer of the zona functionalis—the compacta—with minimal shedding of the spongy later (McLennan and Rydell, 1965). The so-called basal layer has appeared morphologically distinct only during the initial days of the cycle and underwent secretory changes similar to those in the rest of the endometrium (Nogales-Ortiz et al., 1978).

In the rat there is evidence to show that steroid hormones influence the concentration of catecholamines in the uterus. During the estrus cycle, the amount of endogenous uterine adrenaline rises significantly at proestrus (Rudzik
and Miller, 1962; Spratto and Miller, 1968a). During this cycle, the concentration of adrenaline in the uterus is probably related to the level of estradiol in the blood (Yoshinaga et al., 1969; Kalra and Kalra, 1974). The amount of endogenous noradrenaline and its uptake in the uterus do not increase at the same time as the level of adrenaline (Rudzik and Miller, 1962; Spratto and Miller, 1968a). In rat uterus, the adrenaline content increases and the quantity of noradrenaline declines slightly and progressively (Rudzik and Miller, 1962) after administration of oestrogen (irrespective of the stage of the oestrus cycle).

The mechanisms in the control of uterine contractility have been reviewed by Krall and Korenman (1977). It is known that the uterine contractility is regulated by calcium, by steroids, catecholamines and prostaglandins. The molecular mechanisms in the control of myometrial contraction probably involve the $\beta$-adrenergic catecholamines which exert their effect in a variety of tissues entirely by stimulating the enzymatic activity of adenylate cyclase. Other hormones like oxytocin, as well as PG, acetylcholine, angiotensin also influence the adenylate cyclase system.

In the cases so far studied, stimulators of contraction act to inhibit $\beta$-adrenergic-stimulated, but not baseline, adenyl cyclase levels in the uterus. An implication of these results is that agents that increase uterine motility may
do so by suppressing relaxation produced by adenylyl cyclase activation.

Heat or estrus coincides with the greatest development of ovarian follicles. The psychological manifestations of heat are brought about by the female sex hormone, estrogen, which is produced by the ovarian follicles. Complete heat can be brought about by estrogen even in ovariectomized females. It is important to keep this fact in mind, for even though heat is caused by an ovarian hormone, in a sense it is independent of ovarian activity. In intact females, exogenous estrogen causes heat at almost any time during the estrous cycle, and thus heat can be completely divorced from the most important ovarian event, ovulation. This factor in the therapeutic use of estrogen is frequently overlooked in veterinary practice.

In the female guinea pig, a trace of progesterone is necessary before estrogen can cause the female to show full mating response. In the rat, estrogen alone can bring about heat, but less estrogen is required for the full response if the female is pre-treated with progesterone.

When in heat, many animals show a greatly increased activity. Rats in active cages run spontaneously much more at the height of quiet heat than during diestrus or after castration. Spontaneous activity in rats can be increased during the inactive phase by the injection of estrogen (Nalbandov, 1958).
In all animals, the vagina is more alkaline in diestrus and becomes more acid during heat (or during the greatest follicular development). That the change in pH is due to estrogen has been shown by the injection of this hormone into ovariectomized women and cows. The vaginal pH of rats is acid but changes during the cycle and under different experimental conditions as reported by Asdell, (1946). He found that in rats in the diestrus stage, pH was 6.1; at beginning of proestrus it was 5.4; during heat 6.1; in ovariectomized females 7.0 and in ovariectomized female injected with 8 l.U. of estrogen, 4.1.

Changes in vaginal histology during the estrus cycle are found in all mammalian females. The vaginal-smear technique is most useful, however, with animals having short estrus cycles (mice and rats), for in them vaginal histology reflects ovarian events most accurately (Nalbandov, 1958). In animals with longer cycles, such as all domestic animals and women, vaginal changes lag from one to several days behind ovarian changes, and vaginal smears are therefore less reliable indicators of ovarian events. For rats, in which the cycle lasts about four days, very careful comparisons have been made between ovarian morphology and vaginal histology, and the cycle has been broken down into its component parts comprising of 4 stages i.e. Proestrus, Estrus, Metaestrus and Diestrus (Nalbandov, 1958).
Steroidal contraceptives are considered to be the most potent inhibitors of fertility available today. The ability to reliably alter the hypothalamic-hypophysial-gonadal cyclic activity in the normal post pubertal woman has been one of the most successful achievements of reproductive biologists. The use of estrogen/progesterone oral contraceptives and minipill progestin-alone contraceptives has been firmly established through classic experiments with rodents, clinical trials in humans and ultimately consumer use. Continued research is not needed to improve efficacy, which approaches 100%, but continued and improved delivery systems are necessary. How the high degree of efficacy is maintained as the dosages are reduced and refined, also needs to be determined. Reduced sperm migration through the cervix may be critical for one compound, while, interruption of endometrial integrity and implantation for other steroid combinations.

The 1973 research of Barbosa and Ferraz on epidemiologic aspects of human reproduction in Sobradinho shows a high fertility rate for its population; with an average of 4.8 live children per family; the average woman's age was 31.5
(Barbosa and Ferraz, 1973). Contraceptives were used by 45.5% of the couples, and of this group, 76.8% preferred the pill to other methods. Although the pill was the preferred method among a significant percentage of couples, its effectiveness was and is considered questionable by many persons in Sobradinho, including users of the pill. A great deal is now known of the mechanisms by which steroid hormone administration induces temporary infertility in woman and has been the subject of many reviews (Diczfalussy, 1968, 1971; Haller, 1969).

Oral contraceptives act on 1) The hypothalamus-pituitary-ovarian axis; 2) The endometrium, 3) Cervical mucus, and 4) Survival and transport of sperm and eggs. The relative effectiveness of these modes of action varies with the type of contraceptive, dosage of steroids, estrogen/progesterone ratio, endogenous steroid production, estrogen-related abnormal growth, parity and other maternal factors such as obesity and nutritional status (Cohen, 1968; Connell et al., 1967; Diczfalussy, 1968; Drill, 1966; Flowers et al., 1966; Goldzieber, 1970; Maqueo et al., 1970, 1972; Moghissi, 1972; Ryan et al., 1964; Sherman, 1971).

Oral contraceptives under the present formation consist of different combinations of synthetic steroids. The estrogen component consists of either ethinyl estradiol or 17-ethinyl estradiol-3-methyl ether (mestranol). These two estrogens are
different from the parent compound in that an ethinyl group is added at the 17 position. This renders the estrogen orally active. In subjects using an oral contraceptive containing ethinyl estradiol, blood levels of ethinyl estradiol gradually increase from undetectable levels to relatively steady state concentrations 5 days after therapy is begun, with a rapid decrease following cessation of therapy (Longcope and Williams, 1977).

All progestogens presently used in the oral contraceptives are derivatives of testosterone minus a carbon atom at the 19 position. Norethindrone has been in use for more than a decade as a progestational compound in both sequential and combination types. It was thought for many years that norethindrone is converted in the human body to 17 a-ethinyl estradiol (Brown and Blair, 1960). However experiments have failed to substantiate this point (Ryan, 1959; Starka et al., 1966). There is a significant selective uptake of tritiated norethynodrel, which differs from other 19-nor testosterones, by the uterus and other reproductive tract tissues of the rat. Tritiated norethynodrel in women has a relatively long half-life and shows retention in the endometrium (Laumas et al., 1971). Conversion of norethynodrel to norethindrone requires the presence of an oxysteroid isomerase enzyme. The endometrium is very rich in this enzyme. Furthermore, this conversion is much greater in the proliferative than the secretory endometrium (Breuer, 1977).
The antifertility effectiveness of many contraceptive steroids has been thought to reside in their ability to suppress ovulation by interference with the normal interaction of the hypothalamus and pituitary, which results in alterations in the secretion of gonadotrophins (Segal and Atkinson, 1973; Arrata et al., 1980). Secretion of both LH and FSH by the pituitary is reduced in women taking almost any oral contraceptive product.

The beneficial effects of ascorbic acid (AA) feeding to vasectomized rats and those treated with cyproterone acetate has been suggested through several reports from our laboratory, wherein, AA reveals prophylactic effects in restoring the structure, metabolism and secretory activity of the reproductive tissue in male rodents without interfering with the contraceptive purpose of the technique or the drug. The beneficial effects of AA are mediated through its greater utilization, increase in total turnover pattern via the formation of its free radical, monodehydroascorbic acid (MDHA) and charge transfer complex (CTC) formation (Chinoy and Sheth, 1977c, d; Chinoy and Buch, 1977; Seethalakshmi and Chinoy, 1978; Chinoy et al., 1978a-d). Seethalakshmi and Chinoy (1978, 1980) and Chinoy et al. (1979b) have demonstrated that ascorbic acid potentiates the anabolic action of endogenous androgen; and also synergizes with testosterone to increase its anabolic effect. Similar studies on females are lacking.
Ascorbic acid is also known to decrease the amount of blood cholesterol (Ginter et al., 1970, 1971, 1972). These results have added significance as vasectomized animals have a tendency towards fat deposition in rats (Chinoy and Chinoy, unpublished observations), and vasectomized monkeys had greater tendency to develop diet induced atherosclerosis than Sham operated controls fed the same diet (Alexander and Clarkson, 1978). Therefore, the mechanism of action of ascorbic acid will have important implications in the prophylactic treatment of human volunteers following vasectomy, and other contraceptive treatments including steroid contraceptives. However parallel studies in females are very scanty. If ascorbic acid has synergistic action with estrogen and progestogens in enhancing their effect, then it necessarily implies that these anabolic steroids could be administered in much smaller doses, than hitherto used, but along with a known amount of ascorbic acid in the various steroid combinations which are at present used in both females and males.

In the light of the above studies, the present investigation was undertaken to understand the effects of bilateral ovariectomy, hormonal replacement studies as well as ascorbic acid feeding on the histophysicsiology of the uterus of rats, and the synergistic effects with steroids if any, for restoration of the metabolism and structure of the uterus.
2. EFFECTS OF BILATERAL OVARIECTOMY:

Oophorectomy was known to the ancient Egyptians (Finch and Green, 1964). Almost all female mammals will fail to show any sexual behavior after castration unless the appropriate hormones are administered. In the cat (Michael, 1958) and the dog (Leathem, 1938), all trace of sexual behavior disappears after ovariectomy and similar findings have been reported for the guinea pig, mouse, and the rat (Bayrs et al., 1977).

Full receptivity can be induced in the ovariectomized animal by ovarian hormone replacement. An injection of estrogen is followed 24-48 hours later by a single injection of progesterone, and the animals come into heat about 2-4 hours after the progesterone injection in guinea pig, rat, mouse, and hamster. The rat and rabbit show receptivity after estrogen injection alone. However response can be intensified by addition of progesterone (Lisk, 1973).

In the absence of the influence of ovarian hormone the uterus loses virtually all its buffering capacity (Nagakawa, 1935). The development of buffering action in the uterus, therefore, is the result of the change brought about in it by the ovarian hormones. Perfusion of the ovariectomized rabbit uterus with a solution containing estrogen soon imparts a degree of acidity and a buffering capacity which is comparable to that of puerperal uterus (Nagakawa, 1935).
Two or three weeks after ovariectomy, there is no change in the amount of noradrenaline in the uterus (Spratto and Miller, 1968b; Flack et al., 1974), but the quantity of adrenaline is markedly reduced (Rudzik and Miller, 1962; Spratto and Miller, 1968b).

Gay et al. (1970), reported that the ovariectomized rat secretes luteinizing hormone (LH) in a pulsatile way, a phenomenon which has also been observed in other species, for example monkeys (Atkinson et al., 1970; Dierschke et al., 1970), sheep (Butler et al., 1972) agonadal and hypogonadal patients (Root et al., 1972; Santen and Bardin, 1973) as well as healthy subjects (Nakin and Tröen, 1971).

3. EFFECTS OF UNILATERAL OVARIECTOMY:

Unilateral ovariectomy in adult mice and other species leads to compensatory hypertrophy of the remaining ovary (Arai, 1920; Greenwald, 1961; McLaren, 1963; Hermreck and Greenwald, 1964; Brinkley and Young, 1969). This is primarily due to increased gonadotrophin secretion (Grady and Greenwald, 1968; Benson et al., 1969; Welschen, 1970). Gerall and Dunlap (1971) have reported that Unilateral Ovariectomy in the neonatal rat leads to an increase in weight in the remaining ovary within 10-15 days. It was implied that a change in the pituitary-ovarian feedback mechanism was responsible for an ovarian hypertrophy.
The present study was undertaken to elucidate the metabolic changes in ovary and/or uterus of rats during bilateral ovariectomy, hormone replacements, hormone replacement plus ascorbate feeding, and in unilaterally ovariectomized female rats in the light of recent data and with special emphasis on the metabolic role of ascorbic acid.

PART II

EFFECT OF PLANT EXTRACTS:

A number of contraceptive methods are now available to combat the unchecked growth of population. The different methods have varying degrees of failure rate. This failure may result in the birth of an offspring which may be detrimental both to the child and the family. Under these circumstances, post-conceptional therapy is the only remedy to the unwanted conception. A large number of methods, both physical and chemical have been developed to terminate pregnancy.

Attempts to develop antifertility drugs from plants have also been made. The problem underlying the research for natural anti-fertility drugs basically concerns deciding which of the approximately 750,000 species of higher plants should be examined for their potential anti-fertility and abortifacient activity.
Many of these drugs have been screened for anti-fertility activity and few for limited clinical trials. List of such drugs have been compiled by Chaudhury (1966), Dhawan et al. (1976) and Garg et al. (1978). Alcoholic extracts of carrot (Daucus carota) seeds possess estrogenic properties (Sharma et al., 1976). Among rural populations of Rajasthan, dry seeds of carrot Daucus carota, are chewed by women for their reported efficacy in interrupting early pregnancy. However, experimental evidence on the antinidal-tional effect of carrot seeds is lacking, except in rat, where both alcoholic and aqueous extracts of the seed have been reported to prevent implantation (Garg and Garg, 1971). Jacob and Morris (1969) and Jacob and Kaul (1973) found that the postcoital effectiveness of a substance roughly parallels its estrogenicity.

Bitter roots of Aristolochia indica L. an indigenous shrub are reputed to have emmenogogic (Chopra et al., 1956; Kirtikar and Basu, 1935), and abortifacient (Biswa and Ghosh, 1973) properties. It is reported that total crude alcoholic extract of the roots of the plant show 100% interceptive and abortifacient activity (Pakrashi et al., 1976; Pakrashi and Pakrasi, 1977). Similarly, a sesquiterpene extract from the roots of Aristolochia indica was found to possess anti-implantation activity when administered to adult female mice on day 1 of pregnancy. It also showed anti-
estrogenic potency when administered to immature female mice along with estrogen (Pakrashi and Shaha, 1977).

The extracts of the plant of Achyranthes aspera Linn. (Amaranthaceae), an abundant indigenous medicinal herb, showed significant abortifacient effect in mice (Pakrashi et al., 1975) and cent percent abortifacient activity of benzene fraction of the A. aspera (Pakrashi et al., 1975) has been reported in rabbits. The drug is specific as Prakash and Bhattacharya (1977) did not find same abortifacient effect in rat. Abortion may be due to the deficiency in prolactin, growth hormone or pituitary gonadotrophins.

The leaves of Hibiscus rosa sinensis Lonn (Malvaceae), the common garden plant of India are considered to possess emollient, aperient, anodyne and laxative properties. Mixed with the juice of Vernonia cinerea it is used to stimulate expulsion after child birth. Roots are demulcent and used for cough. Contraceptive properties are attributed to the flowers in Ayurvedic literature, Materia Medica and folklores. Batta and Santhakumari (1971); Kholkute et al. (1972); Kholkute and Udupa (1974) have observed anti-fertility properties of this flower. Recently Kholkute and Udupa (1976), reported antiestrogenic properties of H. rosa sinensis in bilaterally ovariectomized immature albino rats. Ethanol extract of H. rosa sinensis was highly effective as an anti-implantation agent (Kholkute et al., 1972). Dixit (1977)
observed that this extract caused degenerative changes in the ovarian tissue.

Daturalactone (DQ₁) isolated from *Datura quercifolia* and its chemically related compounds (DQ, DQ₁, DQ₂H and DQ₁EP) were evaluated for antifertility effects in female albino rats for 1-7 days of pregnancy (Chandhoke, 1977). DQ₁ was the most effective antifertility agent but possessed no antiestrogenic activity. During chemical investigations with *Datura quercifolia* HBK, an annual erect terrestrial herb with a limited range of distribution, a mixture of steroidal-lactones (DQ) was found and on detailed analysis, a novel compound, Daturalactone with structure close to withanolide was isolated from the dried and powdered leaves (Dhar and Raina, 1973). Daturalactone (DQ₁) has been chemically modified and dihydro-daturalactone (DQ₂H), 12-Oxodaturalactone (DQ₂) were prepared. DQ₂ was found to be naturally present in the plant (Dhar and Kalla, 1976).

Methanol extract of whole plant of *Sida carpinifolia* Linn. and chloroform extract of leaves of *Podocarpus brevifolius* altered normal estrus cycle in rats and prevented pregnancy (Khokute et al., 1978), indicating the presence of steroid in the plant. The petroleum ether and methanol extracts of *P. brevifolius* failed to show any significant antifertility effect.

The aqueous extract of dry berries of *Embelia ribes* Burm
was reported to impair the fertility of female mice and rats (Munshi and Rao, 1972; Munshi, 1974) and induce sterility in male mice (Munshi et al., 1972). Embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone) isolated from E. ribes berries has been reported to possess potent antifertility and anti-implantation activities (Radhakrishnan and Alam, 1975; Prakash and Mathur, 1975), but Khalkute et al. (1978) failed to reveal any antifertility activity of Embelin isolated from E. ribes. Arora and Chatak (1971) reported that the aqueous extract of E. ribes when administered from day 1 to 7 of pregnancy at a daily dose of 100 mg/kg showed 85% postcoital antifertility activity.

The antifertility activity of fresh green leaves of Artobotrux odoratissimus Linn. has been confirmed in rats (Chakrabarti et al., 1968; Prakash and Mathur, 1977a, b; Prakash, 1978a). Similarly Prakash (1978b) found antiestrogenic potency of A. odoratissimus extracts in bilaterally ovariectomized immature rats. The activity was dose dependent.

The dried fruits of Piper longum (Piperaceae) and its various extracts were screened for antifertility effect in fertile female rats (Khalkute et al., 1978, 1979). But "Lajjal" an ayurvedic preparation containing P. longum as one of its ingredients failed to inhibit the fertility of rats (Munshi et al., 1977).
Medicinal potions designed as contraceptives or abortifacients are mentioned in ancient writings and in ethnographic literature almost as frequently as magical rites. A good summary statement describing these medicinal brews is provided by Himes: A considerable variety of leaves, herbs and roots, as well as manner of odd and obnoxious substances are pulverized, liquified, and swallowed. It almost seems that the less palatable the substance, the greater the faith in its effectiveness (Himes, 1963). However, most have not been subjected to scientific analysis (Nag, 1968). When tested, some substances have been found to possess potential contraceptive or abortifacient value.

The possible contraceptive or abortifacient value of at least some herbs and other substances used by preindustrial peoples is attested to by the World Health Organizations (WHO) Task Force on Indigenous Plants for Fertility Regulation (Shain and Lane, 1980). WHO is pursuing other projects in the same area-specifically, isolating and characterizing the active agent in substances where preliminary pharmacological data already exists (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 in Lucknow, India has successfully screened
extracts from plants used as antifertility agents, in India. Fourteen of the more than 100 tested demonstrated "greater than 60% antifertility in rats, eight of these were also active in hamsters" (WHO, 1977, 6th Annual Report, Geneva).

Similar antifertility activity has been found in the crude extracts of Haitian Plants. Lastly, preliminary screening of uterotonic activity in 25 Chinese plants has recently begun in Hong Kong. Extracts from three plants have been tested and all show activity (WHO, 6th Annual Report, 1977, Geneva).

Considerable interest has been aroused around the world in Gossypol since the publication of the paper by Chinese scientists (Chinese Med. J. 1978) of its use for fertility regulation in men. Gossypol isolated from Thespesia populnea (Family Malvaceae) is optically active and is strongly dextrorotatory (+) (King and Silva, 1968).

A recent report indicates that 8806 subjects have been treated with gossypol in China (Liu et al., 1981). A large number of derivatives of gossypol, including imino compounds, esters, acetate, formate, and metallic complexes have been prepared but none have shown antifertility activity better than gossypol acetic acid (Wang et al., 1979). There are marked species differences in response to gossypol. Male rat, hamsters, mice, dog, and monkey show varying degrees of sensitivity to gossypol, while rabbits seem to be insensitive
(Cheng et al., 1981). There appear to be strain differences in rats to antifertility action of gossypol (Prasad and Diczfalusy, 1981). Male rats become infertile in 3-5 weeks after daily administration of 15-30 mg/kg of gossypol acetic acid. The onset of infertility seems to be dose related (Xue, 1981; Xue et al., 1980).

No systematic toxicological data on animals with gossypol have been made in some of the Chinese papers (Xue, 1981). Studies with gossypol in male fertility control is available but data on female is scanty.

Recent studies from our laboratory have shown that *Vinca rosea* leaf and *Carica papaya* seed extracts have definite antifertility effects in male rats but little information is available in case of females with respect to the structure and physiology of ovary and uterus. However, the 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 Fransworth (1975), the active principal responsible for anti-implantation effect of papaya seed might be 5-hydroxytryptamine.

In the light of the above studies, the present investigation was undertaken to investigate the effects of plant extracts on Ovary and Uterus of female adult albino rats and also to study the reversibility of the induced effects.
PART III

STUDIES ON EFFECTS OF VARIOUS COPPER-WIRE DEVICES:

In the early 1960s, after more than a century of disrepute, the intrauterine device (IUD) was rediscovered by the medical profession. New interest was spurred mainly by reports of successful clinical studies by Oppenheimer in Israel and Ishihama in Japan (Oppenheimer, 1959; Ishihama, 1959). By the end of 1970s, more than 15 million women were using IUDs, in developed and developing countries (Intrauterine devices, Population Reports, Series B. No. 2. p. 48, 1975). With widespread IUD use, several adverse reactions became apparent. While some complications, such as pelvic infection had been known before and had been responsible for the abandonment of the IUD in the early part of the twentieth century, modern IUDs, bear little resemblance to the first intrauterine devices.

The IUD is still theoretically an ideal form of contraception. Unlike oral contraceptives, it requires no continuing motivation on the part of the user, and its mechanism of action is local, not systemic. Moreover, the IUD offers multitarget mechanisms of antifertility action directed to the sperm, thereby preventing fertilization, to the pre-implantation blastocyst, preventing its implantation; and to the endometrium, indirectly preventing implantation.
Although the precise mechanism of action of the IUD in women is not clear, its contraceptive effectiveness is related to an inflammatory local reaction. The uterine inflammatory response is proportional to the surface area of the IUD. Therefore, an increased surface area of the IUD improves contraceptive performance. However, increased surface area has also been associated with increased frequency of device removal for bleeding and pain (Scommegna, 1980).

Future developments in IUD physical properties may improve retention of the device in the uterus. Recent studies indicate that the more transversely flexible devices have better retention rates. This may imply that the transverse and longitudinal stiffnesses have different effects on expulsion mechanism. Similarly, the addition of anchoring fins similar to those used in the Dalkon shield appears to have improved the retention of a T-shaped IUD. The copper T, Copper 7 are the most common examples of currently available medicated IUDs. Copper devices were shown to cause less bleeding and had a lower expulsion rate and could be used in nulliparous women because of their small size. (Scommegna, 1980; Harper and Sanford, 1980; Rowe, 1981; Hawkins and Elder, 1979; Moyer et al., 1980).

Current studies show that the time span of the effectiveness of the medicated IUD will undoubtedly be extended considerably.
Copper sleeves instead of copper-wire have been added to the T device (T Cu 220 C). This technique permits use of a thicker segment of copper, which releases copper in utero for more than 10 years (Tatum, 1976). Results from clinical trials suggest that there is little difference between the copper T or copper seven and that both are at least equal, and probably superior in performance to the Lippes Loop (Tatum, 1974). There is also some evidence that the copper seven device may be effective for more than 4 years, without need for removal and replacement (Zipper et al., 1976).

In addition, two randomized studies using the T Cu 200 and the Dalkon shield (Moyer et al., 1980), the pregnancy rate and the removal rate for bleeding and for pain was higher with the Dalkon shield, and the continuation rate was higher with the T Cu 200 than with the Dalkon shield.

The major medicated IUDs commercially available and in current use are the Multiload Cu 250, the Cu 200 (Gravigard) the T Cu 200, the progesterone IUD (The Progestasert, 1975), and Copper T 380-A (Rowe, 1981; Moyer et al., 1980). The Multiload Cu 250 has been shown to have the lowest number of pregnancies associated with its use. The net cumulative pregnancy rate for the Multiload Cu 250 was 0.7/100 users after 1 year, in contrast to the T Cu 200, for which the pregnancy rate of 2.9 after one year of use (Mischell, 1975b).
Since the discovery of copper as an antifertility agent, numerous investigators have attempted to maximize the effectiveness of copper for contraception and minimize the side effects. Three parameters have been studied extensively in the clinical use of the IUDs. 1) The placement quantities of copper on the same type of IUD; 2) Different locations of copper on the same IUD; 3) The use of carriers having a different configuration, such as the central versus the lateral location of copper in the uterus. The data from these studies have revealed significant differences in effectiveness, expulsion, and the continuation rates (Moyer et al., 1980).

In a comparative 9-month study, the retention rate for the copper 7 was 17.1% and for the T Cu-200 over 30% (Shaila et al., 1971*). A higher retention rate for the copper T is probably due to the two horizontal arms protruding into the uterus, and the horizontal arms may actually penetrate the endometrium in the area of the cornua (Kamal et al., 1975).

Clinical evaluation of the amount of copper required to produce effective contraception showed that the pregnancy rate with the T IUD without added copper was greater than 18% (Zipper et al., 1969). When copper wire with a surface area of 30 sq mm was wound around the vertical limb of the T,
the pregnancy rate was reduced to 5%. When the surface area of the copper wire was increased to 120 sq mm, the pregnancy rate was further reduced to 2%. Reduction in the pregnancy rate to 1.6/100 women per year was accomplished with the addition of 200 sq mm to the vertical limb of the IUD. Further studies using the T Cu 200 and the T Cu 300 showed that the pregnancy rate was reduced further to 1/100 women per 12 months in conjunction with the T Cu 300 (Timonen, 1976). Additional studies with the T-200 and T-300 revealed pregnancy rates similar to those previously mentioned (Luukkainen and Timonen, 1975). Neither of these devices had copper on the horizontal arms.

In a comparative randomized clinical trial with a T Cu 200 and the T Cu 380, the pregnancy rate ranged from 3.2 for the T Cu-200 to 1.1 for the device with 380 sq mm of copper (Mischell, 1975a).

With the expanding use of the copper T, the safety of the method became an important subject for investigation. The toxicity of copper was studied, particularly the effects of systematic absorption and its teratogenicity in case of accidental pregnancy. Detailed studies in three species of laboratory animals indicated that the copper wire did not produce any teratogenic effects upon developing fetuses. Gestation, parturition, and lactation were not affected by the copper (Tatum, 1977).
The precise mechanism(s) whereby copper exerts its antifertility effect is not known. In most instances it does not prevent fertilization, although copper salts as well as particulate copper are spermatodepressive and, spermicidal (Tatum, 1977; Oster and Salgo, 1977). Chang and Tatum (1970) have shown that the contraceptive action of copper in the rat results in part, from local changes in the endometrial environment, so that the blastocyst cannot implant. They also showed that the antifertility effect does not result from reduced estrogen uptake by the endometrium. The position of copper within the uterine cavity and the exposed surface area of the copper are important in providing antifertility activity (Tatum, 1977). All studies to date indicate clearly that the contraceptive action of copper is due to a local rather than a systemic effect. Although a number of endometrial enzyme systems have been reported to be influenced by the presence of metallic copper (Robles et al., 1972; Chatterji and Laumas, 1974), the significance of these changes in relation to nidation and fetal development is not known. Johri and Dasgupta (1980) found that the activity of monoamine oxidase was stimulated by a copper device, whereas, that of uricase was inhibited and cytochrome c-oxidase activity showed no change.

The effect of copper ions and of copper metal on the rat uterus in vitro has been studied and essentially similar
results have been found with human fallopian tube smooth muscle (Larsson et al., 1976; Oster and Salgo, 1977). Cupric ions at low concentrations (10^{-6}-10^{-5} M) cause the uterus to contract, whereas, at higher concentrations (10^{-4} M) and with prolonged exposure cupric ions cause a decrease in contractile force.

Copper may be acting to stimulate the uterus in a variety of ways. Mechanisms of action for copper-induced uterine contraction involving mercaptyl groups, Cyclic AMP or sodium-potassium ATPase have been considered by Oster and Salgo (1977). The action of copper with Cu-IUD could be through prostaglandin synthesis which in turn could cause pronounced uterine contractions.

Most of the recent efforts to improve intrauterine devices have been directed to increasing their acceptability, rather than their contraceptive efficacy. Copper wire have been added to the T device (Zatuchni et al., 1980). In general, nearly all intrauterine devices have been contraceptively effective, provided they remained in situ and were not dislodged downward into the cervical canal (Shain and Pauerstein, 1980). The expulsion of an intrauterine device is determined by several factors. Of these, the most important is the configuration of the device. Other factors include the size of the IUD, the use of a medicated substance on the IUD, the time during the menstrual cycle at which
insertion is performed, the duration of time that the IUD remains in the uterine cavity following insertion, and the type of inserter.

Copper could produce carcinogens. Copper ions cause milk and edible oils to become rancid by the peroxidation of the unsaturated fatty acids present (Moyer et al., 1980). One of the products of lipid peroxidation is malonaldehyde which is carcinogenic in mice (Oster and Salgo, 1977).

In the light of the above data an attempt was made to study the effects of extra and intra-uterine copper wire devices on histophysiology of female reproductive organs of rats, with a view to developing a suitable and feasible female contraceptive device.

PART IV

EFFECTS OF PROSTAGLANDINS:

The last decade has been the most prolific and stimulating period in the area of prostaglandin research. Prostaglandins are widely distributed in mammalian tissues, although with considerable qualitative, quantitative and species variations.

In the female, the prostaglandins are involved in ovulation and luteolysis, menstruation, spontaneous abortion and labour. The possibility that prostaglandins might be used pharmacologically for fertility control has acted as a great stimulus to research on the role of prostaglandin in female
reproduction (Karim, 1975). Prostaglandin may affect fertility after deposition in the vagina by an action on cervical mucus, vaginal secretion, or by affecting sperm transport in the uterus and fallopian tubes.

Coutinho (1971, 1974) and Coutinho and Maia (1971) have most widely studied the responsiveness of the human fallopian tube and ovary in vivo to prostaglandins, gonadotrophins, steroid hormones, adrenergic drugs, and oxytocin.

The oxytocic activity of some of the naturally occurring prostaglandins on human pregnant myometrium was first demonstrated in vitro by Bygdeman (1964) and in vivo by Bygdeman et al. (1968). Karim and collaborators found elevated levels of prostaglandins in amniotic fluid and in peripheral plasma during spontaneous labour and abortion (Karim, 1966, 1968; Karim and Devlin, 1967; Karim and Hillier, 1970). Shortly thereafter the successful use of natural prostaglandins was reported for induction of labour at term and for termination of early pregnancy (Karim and Amy, 1975).

There are disagreements among the investigators regarding the moment of onset of uterine contractions and the significance of the decrease in plasma steroid levels observed. Csapo et al. (1971) hypothesised that prostaglandins were able to cause a rhythmic uterine activity only after the initial sustained contracture they elicited had altered the functional capacity of the placenta, with an ensuing
decreased circulating progesterone. However, other investigators believe that prostaglandins have a direct oxytocic activity, not mediated by a decrease in progesterone production, and that this is the key to their abortifacient action.

The luteolytic effect of PGF$_2$α was first described in the rat and in the guinea pig and later extended to other laboratory species such as hamsters and the rabbit (Labhsetwar, 1975). Prostaglandins of E series also exert luteolytic effects in the hamster and the rat. In the hamster both prostaglandins E$_2$ and E$_1$ are luteolytic, whereas, in the rat, only PGE$_2$ proved effective. PGE$_1$ was found to be toxic (Labhsetwar, 1975). In general PGE$_2$ is 10 times less potent than PGF$_2$α as a luteolytic agent.

PGF$_2$α has vasoconstrictor action and is present in the uterine tissue of several species. Pharriss implicated the vasoconstrictor property of PGF$_2$α in luteolysis and postulated that PGF$_2$α by decreasing ovarian drainage caused vascular stasis in the ovary leading to the degeneration of corpora lutea. The decrease in the ovarian blood flow was associated with a fall in the plasma progesterone but was not associated with any significant decrease in the renal blood flow. The luteolytic effect of prostaglandins implies that they can exert an antifertility effect by creating progesterone deficiency in the rat.
In addition to PGF$_2$\textsubscript{a}, prostaglandin F$_1$\textsubscript{a} and F$_3$\textsubscript{a} have also been found to exert antifertility effects, although these compounds are somewhat less potent than PGF$_2$\textsubscript{a}. Simultaneous administration of synthetic progestin-provera or progesterone (Labhsetwar, 1975) protected pregnancy implying that progesterone deficiency was the major cause of loss of pregnancy. PGF$_2$\textsubscript{a} has also been reported to exert an antifertility effect in hamsters and mice and rabbits (Labhsetwar, 1975).

PGF$_2$\textsubscript{a} is also active orally as an antifertility agent in the hamster (Labhsetwar, 1975) and the hamster remains the only species other than humans (Karim, 1972), where oral activity of prostaglandins has been demonstrated. The doses required were relatively high. In view of the potent smooth muscle stimulating action of PGF$_2$\textsubscript{a}, it is also possible to argue that antifertility effects result from the direct action of the prostaglandin on the uterus and the resulting loss of the luteotrophic influence of embryo causes luteolysis (Labhsetwar, 1975). In other words, luteolysis may be secondary to antifertility effects.

The mechanism by which PGFs exert antifertility effects in rats and hamsters is not known. Both PGE$_1$ and PGE$_2$ exert luteolytic effects in hamsters as peripheral progesterone levels drop, development of deciduomata is inhibited, corpora lutea atrophy and administration of exogenous progesterone
can maintain pregnancy (Labhsetwar, 1975). However, deficiency of progesterone alone may not be the sole mode of action as the drop in plasma progesterone following treatment with \( \text{PGE}_2 \) is not as marked in hysterectomised hamsters as in intact pregnant hamsters. Thus a contributory action at the uterine level cannot be totally excluded at this time, although the predominant factor appears to be luteolysis (Labhsetwar, 1975).

Sufficient evidence currently exists to suggest that prostaglandins (PGs) are involved in the process of ovulation. As the time of ovulation approaches in response to gonadotropin treatment, the prostaglandins \( \text{E} \) and \( \text{F} \) increase markedly within the Graafian follicles of both the rat and rabbit. Moreover, in rabbits, \( \text{PGF}_2^\alpha \) administration results in accelerated egg transport (Labhsetwar, 1975).

The present investigation was undertaken in the light of the above data to highlight the effect of \( \text{PGE}_1 \) and \( \text{F}_2^\alpha \) treatments on the physiology of ovary and uterus, and their histology, biochemical parameters including ascorbic acid turnover pattern.

**PART V**

**VITAMIN C DEFICIENCY IN GUINEA PIGS:**

The only laboratory mammals in which ascorbic acid (AA) deficiency can be induced are guinea pigs, since like primates they cannot synthesize the vitamin. The AA deficiency is
reflected in levels of ascorbic acid in plasma, liver and spleen (Anthony et al., 1979). Additional impact of AA deficiency was seen on loss of weight and reduced food intake.

The role of ascorbic acid (AA) in the male reproductive system has been elucidated (Chinoy, 1978). Many workers have emphasized its importance in the biosynthesis of sex hormones (Biswa and Deb, 1965; Datta and Sanyal, 1978; Agrawal and Laloraya, 1977, 1978; Chinoy et al., 1980a). Paul and Duttagupta (1978) postulated that vitamin C therapy to underfed rats improved the adverse effects of diet restriction on sex accessory gland secretions, while it induced a toxic effect on the ad libitum fed rats. Hanck and Weiser (1973) have postulated that in scorbutic guinea pigs, the intake of megadoses of ascorbic acid was well tolerated and resulted in no adverse effects, but rather physical and biochemical status were improved.

In the deficiency state, the plasma levels of AA were unmeasurably low. The male guinea pigs are known to succumb to AA deficiency sooner than females (Harman, 1950; Odumosu and Wilson, 1973). In male guinea pigs, the deficiency of vitamin C has been shown to be accompanied by atrophied testes (Chatterjee, 1967), impaired androgen synthesis (Belavady and Banerjee, 1954), arrested spermatogenesis (Biswa, 1967), reduced sperm motility, accumulation of
l lipid and decrease in androgen sensitive metabolism of testis, epididymis and accessory glands together with changes in their histoarchitecture (Seethalakshmi and Chinoy, 1976; Chinoy et al., 1982). Furthermore, studies from our laboratory have shown the alterations in the morphology of epididymal and vasal spermatozoa of the scorbutic guinea pigs, wherein the abnormalities were mainly observed in the head and tail region thereby rendering the sperms immotile and non-fertile (Chinoy, N.J., Chinoy, M.R., 1979; Chinoy et al., 1982).

Clinical and experimentally induced vitamin C deficiencies, both have been found to be important factors in the etiology of abortion. By completely blocking progesterone synthesis, not only in the ovary, (which in the human species would be of little consequence), but also in the placenta and the adrenal, vitamin C deficiency leads to complete failure of the entire hormonal system responsible for protecting gestation. By depressing adrenal function during pregnancy, vitamin C deficiency may equally well play an important role in the genesis of some pathogenic conditions of pregnancy (Botella-Ilusia, 1973).

Data are available on mechanisms for placental transfer of ascorbic acid from maternal to fetal circulation (Kaminetzky and Baker, 1980). Ascorbic acid exists in maternal blood as dehydroascorbic acid and L-ascorbic acid. The placenta is freely permeable to dehydroascorbic acid but not to ascorbic acid (Hafez, 1980). The fetus is supplied
transplacentally only with dehydroascorbic acid; the placenta
and/or fetus then converts it to ascorbic acid. Ascorbic
acid and not dehydroascorbic acid accumulates in the fetus
(Raika, 1958).

Fatigue has long been recognized as a characteristic
symptom of scurvy in man (Crandon et al., 1940). Basu and
Biswas (1940) claimed that ascorbic acid improves muscular
function, and its metabolic significance for muscle metabolic
has been elucidated by Chinoy and Kshatriya (1977). It has
been shown (Lloyd and Sinclair, 1953) that in scorbutic
condition, the smooth muscle reaction to histamine and
adrenaline was markedly reduced.

In the light of the above data, the present study was
undertaken to ascertain the significance of ascorbic acid
in reproduction of guinea pigs, especially with reference
to changes in their ovarian and uterine histo-physiology
and cyclicity.