REVIEW OF LITERATURE
The discovery of clomiphene (1-p-diethyl amino-ethoxyphenyl-1,2-diphenyl-2-chloroethylene), an analogue of non-steroidal estrogen, chlorotrianisene (TACE) has established a most challenging moment during the search of suitable antifertility compounds in the arduous field of reproductive physiology.

Clomiphene citrate (SER-41) is also unique, since it has ovulation inducing propensities in anovulatory sterile and irregularly menstruating women (Greenblatt, 1961; Seyer, 1963). Besides this, clomiphene and its isomers have wide spectrum of pharmacodynamic effects in the laboratory animals, thereby furnishing a great promise as potent antifertility compounds. Amongst the multiple effects of clomiphene compounds, stimulation of the hypothalamic-pituitary axis with a resultant release of FSH/LH (Roy et al. 1963; Chatterjee et al. 1974; Gupta et al. 1974), inhibition of gonadotrophine secretion (Holtham, 1960; Hollinger, 1971), stimulation of steroidogenesis by acting directly at the ovarian or luteal level (Smith et al., 1963a; Hammerstein, 1967), inhibition of blastocysts Implantation (Schlogh & Meyer, 1969), anticytokic effect (Segal & Nalson, 1961; Prasad et al., 1965), are well documented. Moreover, clomiphene does not behave like androgen, progesterone or the corticoids (Greenblatt et al., 1961). The antifertility faculty of clomiphene in several laboratory animals have recently been explored. However, its mechanism of action so far proposed is seemed to be puzzling. Two primary theories have been put forward, i.e. the first theory claims that clomiphene acts on the hypothalamic-pituitary complex (Greenblatt, 1961; Roy et al., 1963; Thompson & Hollinger, 1965; Hyne et al., 1966; Bardin et al., 1967; Jacobson et al., 1968 and Peterson et al., 1968) with resultant release of gonadotrophine.
FSH (Bardin et al., 1967; Boyar, 1970) and LH (Bardin et al., 1967; Taubert et al., 1970). Igarashi et al. (1967) reported that clomiphene could cause a significant rise of FSH and LH titers in the plasma of mature rats and claimed the hypothalamus as the principal site of action of clomiphene. This view is also supported by Baler & Taubert (1969) who have shown that clomiphene (100-300 µg) in estrogen-progesterone blocked rat causes a significant decrease in hypothalamic FSH-Rf content with concomitant increase in plasma FSH. Very low dosage (1-100 µg/kg) of it could also increase gonadotrophin secretion as evident by gain in ovarian weight, increase in the number of corpora lutea and gonadotrophin-releasing factors in the hypothalamus (Koch et al., 1971). By using different experimental model, Kastin et al. (1970) and Schneider et al. (1969) have found an increase in LH secretion after clomiphene administration. Their observation strongly suggests that in their test animals removal of the negative feedback by estrogen is not necessarily involved in stimulating gonadotrophin secretion by clomiphene. Yan-Samuel (1970) has treated patients having polycystic ovary syndrome for 3-5 days with clomiphene citrate (50-100 µg/day) and observed a rise in LH and FSH secretions during the treatment schedule. However, in oophorectomized estrogen-progesterone blocked rats Taubert et al. (1970) have failed to detect any positive effect of clomiphene (10 µg or 50 µg) on the pituitary LH levels; clomiphene, on the other hand, has been reported to inhibit the sequence of compensatory hypertrophy of the remaining ovary in the adult hemispayed rats (Dano et al., 1972). Moreover, Döcke & Doerner (1971) are of opinion that clomiphene may prevent the estrogen-induced sensitization of the anterior pituitary to hypothalamic gonadotrophin releasing factor. Boyar (1970) again
claims that clomiphene at a lower dose level of 0.3 mg/kg bw for 10 days causes decrease in gonadotrophin secretion. The low dose effect of clomiphene is similar to that observed in women, which is believed to result from clomiphene's ability to compete with natural estrogen at hypothalamic-pituitary receptor sites.

Clomiphene-induced ovulation and formation of corpora lutea in rats by a direct hypothalamic stimulation at a dose level of 2 mg/kg bw for 5 days in post-natally androgenized female rats, and a 10 mg dosage for 5 days in rats having lesion at the suprachiasmatic nucleus and pre-optic area suggest that clomiphene possibly produces its effect in the area of ventromedial and infundibular nuclei (Röcke, 1969). Röcke (1969) again reported that ovulation could be induced in rats in which a chronic state of anovulation is being set up by administration of testosterone propionate on day 3 of postnatal life, by continuous light or by an electrolytic lesion at the level of the median anterior hypothalamus. Hohlweg & Mayer (1970) have induced corpus luteum formation in the immature female rats by a single sc injection of 25 to 75 mg/day of clomiphene. Newton & Dixon (1971) have given clomiphene (50-200 mg/day) for 5 days in oligomenorrheic women with secondary amenorrhea and observed an increase urinary LH levels before any increase in total estrogen levels.

Santon et al. (1971) have also shown that clomiphene (100 mg/day for 7 or 51 days) in normal men increases mean serum levels of FSH, LH and testosterone. Christiansen (1972) has also confirmed that clomiphene citrate (50 mg/day for 30 days) in infertile men increases urinary levels of total pituitary gonadotrophins-FSH and LH, with no side effects as a result of the drug administration. Nagata et al. (1972) have reported an increase of 2.5 fold of blood...
FSH and LH after the administration of clomiphene (100 mg/day for 5 days) to adult males. According to Ishizuka et al. (1973) cyclofenyl and clomiphene increased LH excretion in urine in healthy subjects and women with various anovulatory states. Mazzi et al. (1973) have claimed that clomiphene citrate (100 mg/day for 7 days) increased urinary FSH and LH. Further, clomiphene citrate of 150 or 330 µg in female rats shows evidence of stimulation of pineal body. But, this pineal function appears to be suppressed by still larger dosage. Martinez Montes & Gentili (1971) have induced prolonged estrus, precocious vaginal opening in female rats, that have been treated with clomiphene citrate (50-500 µg) on days 3-5 after birth. They have further added that clomiphene in suspension has no effect on ovarian structure, but when administered at 50 µg dose level in solution state caused an increase in body weight associated with polycystic ovaries. They are of opinion that clomiphene-induced hypothalamic activity is comparable to estradiol benzoate. Miechi et al. (1975) also have shown that clomiphene citrate increases blood serum LH and testosterone levels in normal fertile men and also in Parkinsonism patients (Delitala et al., 1976). Again, Naftolin et al. (1973) have claimed that an oral dose of 100 mg clomiphene/day in men for 8 days increases serum titers of LH and testosterone on day 4 of treatment but does not affect the frequency of LH pulses. A significant increase in serum concentration of LH, FSH and testosterone has been, however, recorded by Kampmann et al. (1976) after 8 days and with a further increase when measured a week later. The effect of clomiphene citrate (100 mg daily for 10 days) on the gonadotrophic response to synthetic LH-RH has been investigated by D'Ambrosio et al. (1976) in normal males. Clomiphene increases the baseline levels of LH or FSH after a few days of treatment. Babeljuk et al. (1972) however, reported that clomiphene...
clomiphene in sheep depresses the stimulatory effect of LH-RH on the secretion of LH. Moreover, the FSH and LH stimulation of LH-RH during late follicular and mid luteal phases in women has also been investigated to be abolished following clomiphene medication (Wang et al., 1975).

In man, clomiphene induces massive ovarian hypertrophy (Southam & Janovski, 1962) due to augmented release of FSH (Roy et al., 1963). Furthermore, it appears to suppress the ovulation inhibitory action of estrogen (Pincus, 1965). Conversely, clomiphene can also induce ovulation in pseudopregnant rats (Watnick & Nori, 1968; Taubert et al., 1969), an effect which is thought to depend on the estrogenic activity of the compound. The rise in total gonadotrophins, FSH and LH excretion have also been reported by Greenblatt et al. (1962), Heinrichs & Zander (1964), Mellinger et al. (1963), Bettendorf et al. (1965) and Harkness et al. (1964, 1965) following clomiphene therapy ranging from several days to few weeks. Schally et al. (1970) have found that clomiphene and its cis- and trans-isomers at low dosage of 15 µg per day for 3 days appear to stimulate LH release while Nagel, Bailer and Taubert (1970) have carried out investigation on the effect of cis- and trans-isomers of clomiphene upon the release of FSH in the oophorectomized, estrogen- proges­terone-blocked rats. The data obtained clearly reveal that cis-clomiphene is effective at a dose level of 150 µg/rat to lower the hypothalamic content of FSH-RH and of pituitary FSH content. Higher dosage of cis-isomer in the identical situation shows just the reverse effect. However, administration of trans-clomiphene of 25 to 900 µg/rat found to be completely ineffective over the entire dosage range. Faiman & Ryan (1968) similarly observed that oral administration of cis-clomiphene to men is followed by an elevation of
plasma FSH, while the trans-isomer has no effect. Singh et al. (1973a) have shown that depletion of pituitary LH is marked by the injection of trans-clomiphene than in those treated with the same dose of the cis-isomer; however, the depletion of pituitary FSH is found to be identical with both the isomers. The cis- and trans-isomers of clomiphene citrate exert their effects by acting on the hypothalamus and that the trans-isomer being more estrogenic than the cis-isomer.

Taubert & Juergenson (1972) have shown the action of clomiphene on the hypothalamus, adenohypophysis and ovary and suggested its practical importance in the treatment of anovulation, luteal phase defects and in determining the integrity of the hypothalamic-pituitary axis. Cooke and Learner (1971) have injected clomiphene citrate of 2 mg/kg bw for 5 days to postpubertal androgenized rats and could induce ovulation in 48% of the animals. Again clomiphene (10 mg/kg for 10 days) in rats with lesion in the supra-chiasmatic nucleus or medial preoptic area could induce corpora lutea in 100% of the test animals. Implantation of small dosage of the compound in the hypothalamus or anterior-hypophysis indicates that clomiphene exerts its effect in the area of the ventromedial and infundibular nuclei. Clomiphene per oral route over 3-5 weeks to anovulatory sterile women causes ovulation in all the patients of whom 40% conceived (Boqui & Hammerstein, 1969). Von-Elemendorff and co-workers (1968), Roland (1970) and Sharf et al. (1971) have demonstrated that concomitant treatment of clomiphene with HCG induces ovulation in greater percentage of the anovulatory subjects, including those who have resistance to clomiphene alone. Radwanska and his associates (1974) have shown that treatment with HCG, clomiphene or clomiphene and HCG lead to 2-fold
increase in plasma progesterone, however, clomiphene and HCG treatment being the most effective for frequency of conception. In the anovulatory patients, ovulation induction successfully results with clomiphene or clomiphene and HCG. Defective ovulation and luteal insufficiency as detected by plasma progesterone determination, probably play a significant role in infertility and can be corrected in many cases by the preovulatory stimulation of ovarian functions. Since treatment of the sterile and anovulatory women with prolonged clomiphene therapy (50-100 mg/day) shows signs of ovulation, followed by pregnancy in some of the patients (Sharf et al., 1971). Bishop (1970) has also been successful in inducing ovulation with clomiphene by stimulating anovulatory ovary to secrete estrogen. In contrast to its effect in human, clomiphene fails to induce luteinization in the ovaries of rhesus monkeys but it found to cause a marked follicular proliferation associated with estrogenic secretion (Aguilar Olivan and co-workers, 1973). In this respect trans-clomiphene is less effective than clomiphene but the cis-isomer shows little, if any, ability to facilitate ovulation (Ying-Shae-Yee & Mayer, 1972).

Recently it has been documented that high doses of estrogen fail to interact with the ovulation inducing faculty of clomiphene. In all ovulatory cycles so far studied, the preovulatory estradiol peaks have been found to be significantly higher than those in normal cycles (Taubert et al., 1976). To establish the site and mechanism of ovulation inhibiting efficacy of clomiphene, Böcke (1971) has suggested, on the basis of his experimental data, that clomiphene has a definite antiestrogenic effect at the level of the anterior pituitary.
Kato et al. (1968) have recorded that administration of clomiphene causes a marked dose dependent depression in the uptake of tritiated estradiol by anterior pituitary in ovarioctomized rats. A strong antiestrogenic activity of clomiphene has also been established by Van Maanen and co-workers (1961) and Roy and his associates (1964). It is evident that clomiphene prevents the entry of estrogen into the anterior pituitary (Roy et al., 1964b). Cis-clomiphene shows to have an antiestrogenic influence on the vaginal cytology, cervical mucus and endometrium. Moreover, cis-clomiphene appears to be more potent than that of trans-clomiphene in inducing ovulation (Greenblatt et al., 1971). Furthermore, the orally administered clomiphene has been shown to inhibit uterotrophic action of stilbestrol in castrated rats (Matnick et al., 1969).

While studying the sexual behavioral pattern of ovarioctomized female rats, Ross and his associates (1973a, b) have recorded that trans-clomiphene (2.5 mg sc) could induce the sexual behavior in the absence of estradiol benzoate, however cis-clomiphene does act quite differently and blocks the estradiol-benzoate-induced sexual behavior.

Prasad and Kalra (1967) presume that the antifertility role of clomiphene depends on the increase in uterine motility, expulsion of the blastocysts or an antiestrogenic or antihistaminic action which could prevent the pre-implantation changes in the uterus. The same school of investigators have further studied the uterine sialic acid content in estradiol and clomiphene-treated rat during delayed implantation and have found that estrogen or clomiphene induces a sharp increase in sialic acid concentration within 6 hr of treatment. Pretreatment with clomiphene or concomitant treatment with estradiol inhibits
the estrogen-induced increase in sialic acid content (Rajalakshmi et al., 1970). Kahwanago and associates (1970) have shown that uptake of $^3$H-estradiol in the pituitary and hypothalamic receptor have been consistently inhibited by clomiphene. Vaalukaitis and his co-workers (1971) are again of the opinion that clomiphene apparently competes with estrogenic receptor sites in the pituitary gland or hypothalamus.

Clomiphene citrate, ICI-46474 or U-11100A has also been found to inhibit the binding of 17-$\beta$-estradiol by cytosol fractions of human uterus (Wyss et al., 1968; Haehnel et al., 1973). Schulz (1971) has claimed that after a single sc injection of 0.5 mg cis-clomiphene/100 g bw a rapid increase of $^3$H-uridine incorporation into uterine RNA occurs, followed by an increased incorporation of $^3$H-leucine into uterine proteins. Trans-clomiphene (0.3 mg/100 gm bw) has been found to have a similar effect on RNA and protein synthesis. The effect of both these isomers on uterine protein synthesis reached a maximum at 12 hr which is again maintained for 36 hr. Morris et al. (1976) have shown that following the administration of cis-clomiphene citrate to rats, cytosolic estrogen receptor concentrations reduces to 3% in the pituitary and 5% in the uterus, while receptor concentration in the amygdala and hypothalamus remain unaffected. Estradiol-17-$\beta$ increases receptor concentration by 55% in the uterus and stimulation of gonadotrophin release following cis-clomiphene may be effective via the pituitary pathway. In support of the antiestrogenic potency of clomiphene Weisberg (1975) has shown that clomiphene citrate, an antiestrogenic agent, antagonises the formation of 3'-5'-cyclic AMP elicited
by 17β-estradiol and diethylstilbestrol whereas it has no stimulatory effects on 3'-5' cyclic AMP formation itself. Apparently binding of estrogenic compound to cytoplasmic receptor sites in the hypothalamus is required for subsequent stimulation of 3'-5' cyclic AMP formation. Potest and Bo-Walter (1971) have administered 50 µg of clomiphene citrate concomitantly with estradiol dipropionate (1.0 µg) and observed that estrogenicity of clomiphene on myometrium is less than that of estradiol alone, however, the luminal epithelial cells with its glycogen content increased strikingly after clomiphene therapy but not after estradiol treatment. Thus the effect of clomiphene on the luminal epithelium may be either a unique action of the drug or an abnormal response of the tissue similar to that reported for high dosage of estradiol.

Antifertility faculty of clomiphene has been evaluated in numerous experiments. Jacob et al. (1969) have reported that like different estrogenic compounds clomiphene (2-15 mg/kg) on days 1-3 postcoitum in rabbits appears to inhibit blastocyst implantation. However, as per Andrade et al. (1972) clomiphene actively affects the development of blastocysts when observed in in vitro preparation, but normal fetal development has been recorded when the blastocysts are transplanted to recipient only after 4 hr of incubation with 0.5 µg clomiphene citrate. The zygotic effect of clomiphene has been possibly proposed for the first time by Holtkamp and his associates (1960, 1961) and later followed by Segal and Nelson (1961) and Prasad and his colleagues (1965). However, utilizing the fascinating blastocyst transfer technique and recovering live fetuses from the recipients, Staples (1966) has strongly challenged the idea of blastocidal effect of clomiphene and proposes its antifertility effect by altering the endocrine profile of the maternal organism.
Cloalphene has been shown not to have any teratogenicity in pregnant rats except causing a low percentage of fetal mortality (Suzuki, 1970). Recent observation of Hashizume and his associates (1976) reveals that cloalphene at a dose of 2.5 mg on various days between 15 and 21 of pregnancy causes parturition in rats.

The direct action of cloalphene on steroidogenic tissue has drawn the attention of several investigators. Smith and co-workers (1963 a, b) believe that cloalphene acts directly by interfering with the enzyme systems involved in ovarian steroidogenesis. Aromatization of testosterone by placental microsomes with a resultant increase in 1,6-fold estrogen has been documented. Stimulation of 3β-ol-dehydrogenase–Δ5 isomerase activity in slices of human corpora lutea by cloalphene has also been noticed by Hammerstein (1967). However, at a higher dose level, cloalphene has been found to block the steroidogenic enzyme systems. The stimulatory effect of cloalphene at the ovarian level is proposed to be accompanied by a better utilization of HCG or LH (Hammerstein, 1968, 1969 a, b). Stimulation of side chain cleavage of cholesterol and progesterone in testicular homogenates by cloalphene has also been evident (Carlstrom et al., 1974). The cloalphene-induced luteolysis and fetal resorption has been shown to be associated with decreased activities of glucose-6-phosphate dehydrogenase, malate dehydrogenase and ATP citrate lyase of corpus luteum, but not 6-phosphogluconate dehydrogenase, 3β-hydroxysteroid dehydrogenase and pyruvate kinase activities (Okazaki, 1975). The idea of an increased ovarian sensitivity to LH by cloalphene has been supported through several experimental data (Mayfield and Ward, 1966; Dahl, 1970, 1971).
Singh and his colleagues (1973b) have reported that trans-clomiphene seems to be more potent in adult male rats than the cis-clomiphene in terms of a decline in weight of testis as well as accessory reproductive glands. However, the secretory activity of the accessory glands, the motility and fertilizing capacity of spermatozoa and moreover, the reproductive behavior of the rat, are all found to be decreased by both the isomers of clomiphene. Moreover, treatment of cis-clomiphene for 60 days or chronic treatment with trans-clomiphene causes decrease in spermatic acid levels in caput and cauda epididymis along with the structural regression of the tissue (Rajalakshmi et al., 1970).