RESULTS AND COMMENTS
Injection of clomiphene, cis-clomiphene or trans-clomiphene on day 5 of pregnancy was resulted in complete resorption of the embryonic swellings when examined on day 14 of pregnancy, while the embryonic swellings in the vehicle-treated controls were weighing 516.4 ± 13.8 mg. A significant reduction in ovarian weight was, however, recorded in the animals which had clomiphene (51.9 ± 3.6 mg; P<0.01) or its cis-isomer (51.3 ± 2.5 mg; P<0.01), while the individuals which had trans-isomer did not show any significant difference in ovarian weight compared to the vehicle-treated controls. Moreover, none of the compounds was found to be effective in altering the luteal weight of the experimental animals (Table 1), however, ovarian polyfollicular state was evident in all the cases following the treatment of either clomiphene or its cis- or trans-isomer.

Studies on women subjected to clomiphene medication clearly demonstrate an increased excretion of gonadotrophins (Greenblatt et al. 1962). Further, the ovarian polyfollicular state in our experimental rats is in agreement with the observations of Igarashi et al. (1967), Kato et al. (1968) and Baier & Taubert (1969) that clomiphene and its isomers act at the level of the hypothalamic-pituitary complex and cause an augmented blood titer of FSH and/or LH (Greenblatt, 1961; Roy et al. 1963; Thompson & Bellinger, 1965; Boyar, 1970; Taubert et al. 1970; Vanderberg et al. 1973; Singh et al. 1973 a,b; Yao & Bettendorf, 1973). As a result of which increased production of estrogens of ovarian origin occurs (Roy et al. 1963). The estrogen-induced augmented release of LH (Rennels & O'Steen, 1967) has been shown to have some luteolytic effect (Rothchild, 1965, 1966; Spies et al. 1966; Greenwald & Johnson, 1969; Yoshinaga et al. 1972; Chatterjea, 1973; Pal, Thesis, 1976). This collective information, therefore, naturally raises
the question whether the antifertility efficiency of clomid or its isomers is the consequence of an excessive liberation of endogenous gonadotrophins specially that of LH. To justify such a possibility experiments were modified by using a concomitant regimen of reserpine, a consistent blocker of pituitary gonadotrophins release (Khazan et al. 1960; Chatterjee & Harper, 1970; Chatterjee et al., 1974) concurrently with clomid, its cis- or trans-isomer as per the schedule (Table 2).

At sacrifice, on day 14 of pregnancy it was found that reserpine at our experimental dose level could mitigate the antifertility influence of clomid, maintaining the growth status of embryonic swellings, and corpus luteum statistically parallel to pooled controls excepting that of ovaries- \( P < 0.01 \) (Table 2). Thus, reserpine along with clomid could counteract the detrimental influence of the compound which is in good agreement with that of Gupta et al. (1974) who also obtained identical results in rats during the later half of pregnancy. Conversely, the detrimental influence of cis- or trans-isomer of clomid on gestation could not at all be combated by the addition of reserpine concurrently with either of the agents. From the present observation it is justified to assume that the antifertility faculty of cis- or trans-clomid is not possibly resulted from the LH-stimulated luteal demise.

The preceding experimental findings, therefore, suggest that the antifertility faculty of cis- and trans-isomers of clomid at our selective dose level is not perhaps the consequence of an induced high titer of endogenous gonadotrophins. The ovarian polyfollicular state as evident in the preceding experiments may be associated with functional luteolysis leading to progesterone deficiency, since X-irradiation which destroys ovarian follicles is reported to be
To clarify the luteal hormonal deficiency as the possible cause of clomiphene or its isomer-induced failure of pregnancy, rats were sorted into several groups and the treatment of clomiphene or its cis- or trans-isomer concomitantly with progesterone regimen was followed (Table 3). Progesterone (hydroxyprogesterone-capsrate) was given sc as a daily injection (5 mg/rat) from day 5 through day 13 of pregnancy in a volume of 0.2 ml of the vehicle, and the animals were sacrificed on day 14 of pregnancy.

Progesterone replacement in the clomiphene-treated pregnant rats was reflected by a successful maintenance of pregnancy, the embryonic swellings were, however, weighed less to that of the controls (496.6 ± 18.9 mg; P < 0.10), while weight of the ovaries and CL were not statistically different (Table 3). Conversely, an identical replacement schedule of progesterone along with cis- or trans-clomiphene was found to fail pregnancy maintenance, since the drug schedule led to complete resorption of the established embryos with the sign of vaginal bleeding between days 13 and 14 of pregnancy (Table 3).

On the basis of the above findings it was reasonable to assume that clomiphene-induced ovarian malfunctioning possibly leads to progesterone deficiency. While, the failure of progesterone replacement in preventing the pregnancy wastage caused by isomers of clomiphene suggests that the cis- or trans-isomer of clomiphene may manifest the detrimental effect in a different fashion, rather than restricting the endogenous availability of progesterone. Thus, the negative result following progesterone replacement in the cis- or trans-clomiphene-treated rats once again enticed
us to believe that effectiveness of the isomers of clomid as antifertility agents may be due to the development of some altered utilization capacity of progesterone at the uterine level.

It is evident that both cis- and trans-isomers could compete with the estrogen receptor sites (Rub et al. 1974; Schulz et al. 1973, b), and a competition between estrogen and progesterone is also known to occur at the uterine receptors level. To have a decisive answer on the possible competitive attitude between the isomers of clomid and the progesterone at the uterine level, experiments were conducted on pseudopregnant rats.

Pseudopregnancy was induced on regulatory cycling females by sterile mating. The pseudopregnant rats were subjected to bilateral castration on day 4 evening and uterine traumatization on day 5 by using classic method of De Feo (1963) for inducing decidual cells reaction (DCR). Progesterone at a dose of 5 mg was continued from the day of operation through day 9 of pseudopregnancy. Animals were sorted into groups as per the treatment schedule (Table 4). On sacrifice (day 10), uteri were removed and split at the bifurcation, the adhering tissues were trimmed off, and each cornua weighed and compared with the vehicle-treated controls.

Table 4 shows that the degree of DCR in the clomid-treated experimental animal (1090.7 ± 87.3 vs. 103.3 ± 9.9 mg; P < 0.01) was almost identical to controls (1150.3 ± 111.4 vs. 95.7 ± 8.5 mg; P < 0.01). However, cis- or trans-clomid in an identical situation completely abolished the uterine response in the development of DCR (Table 4), indicating the existence of direct antagonism between cis- or trans-clomid with the progesterone at the uterine level.
On the basis of foregoing experimental documentation it is reasonable to believe that the isomers of clomid could possibly compete at the uterine receptors level and make the uteri almost refractory to progesterone which may in turn affect the endocrine support of pregnancy or LHR. However, to confirm the last possible question of whether the hazardous effect as exerted by cis- or trans-clomid is exclusively localized at the uterine level or it is a joint venture of the same compounds at the level of uterus and the pituitary-ovarian axis, cyclic animals were subjected to surgical extirpation of uterine tissue (bilateral hystectomy) and made pseudopregnant on sterile mating. It was found that pseudopregnancy continued in the hysterectomized control rats for 18-20 days. Cis- or trans-clomid schedule on day 5 of pseudopregnancy was found ineffective, since the animals which had either cis- or trans-clomid exhibited an identical length of pseudopregnancy 18 – 20 days as found in controls.

The series of experimental findings of this thesis, therefore, revealed that clomid at its antifertility dose level of 3 mg/kg bw mainly develops deficiency of ovarian progesterone, however, cis- (2 mg/kg) or trans-clomid (10 mg/kg) as antifertility agent has been found to develop progesterone deficiency by altering either its uptake or the utilization capacity by the uterine tissue.