CHAPTER VII

PRE-AND POSTIMPLANTATIONAL EFFECT OF BROMOCRYPTINE DURING PREGNANCY
INTRODUCTION

Prolactin is one of the pituitary hormones which is essential for the maintenance of the corpus luteum and its secretory activity during early pregnancy in rodents. It has long been appreciated that rodent corpora lutea could be maintained by either coital stimulation (Long and Evans, 1922) or injection of pituitary extracts containing Prl activity (Astwood, 1941; Mac Donald and Greep, 1969). An overwhelming evidence indicates that the mating stimulus induces the release of Prl which in turn stimulates the corpora lutea of the cycle and prolongs the ability of these structures to secrete progesterone (Neill and Smith, 1974; Smith et al, 1975; Smith et al, 1976; Smith, 1980). If the mating is sterile or the cervical stimulation copulomimetic the surges of Prl continue for 12-13 days and this period is known as pseudopregnancy. However, in case of fertile mating the Prl surge continue only for 10 days (Smith and Neill, 1976; Yogev and Terkel, 1978). Prolactin levels are low and unchanging for the remaining period of pregnancy. These surges of Prl are responsible for the maintenance of the corpus luteum during early pregnancy (Morishige and Rothchild, 1974) whereas, a luteotrophin secreted from the rat's placenta during the second half of pregnancy maintains P secretion during the latter part of the
gestation period (Kelly et al, 1976). This early termination of pituitary Prl surges during pregnancy on day 10 as compared to day 12 in pseudopregnant rats is correlated with the ability of the rat placenta to secrete a placental lactogen (rPL) which assumes the luteotrophic role of the pituitary (Smith and Neill, 1976; Yogev and Terkel, 1978; Voogt, 1980).

Blockade of Prl release on or before day 7 in intact pregnant rats terminates pregnancy (Sheleshyak, 1955; Kisch and Sheleshyak, 1968; Clemen et al, 1969; Babu and Vijayan, 1983). An ergoline like compound, D-6-Methyl-8-cynomethyl ergoline was also shown to terminate pregnancy when injected on or before day 7 but not on day 9 (Rezabek et al., 1969). In another study Be administration during the first five days of pregnancy lead to inhibition of blastocyst implantation (Muller et al, 1980). Inhibition of Prl secretion during second half of gestation did not have any effect because the rPL comes into being by day 9 which maintains the P secretion from the corpus luteum.

Glucosamine 6-phosphate synthase, a glycoprotein synthesizing enzyme, has been demonstrated to increase during early pregnancy (Rukmini, 1981). Hence, in this study the pre-and postimplantational administration of
Be on uterine glucosamine 6-phosphate synthase levels have been evaluated.

MATERIALS AND METHODS

Adult female rats displaying regular 4 day cycles were mated with males of proven fertility on the evening of proestrous and the vaginal smears were observed under a microscope on the following morning. Day one of pregnancy is designated as the day on which the spermatozoa were observed in the vaginal smear. Bromocryptine was injected sc in three groups of animals in doses of 200 yg/100 g body weight dissolved in 100 l.l (of 1:1:2 ratio of propyleneglycol:ethanol:saline on days: (1) 1-5, (2) 9-15 and (3) 14-20 of pregnancy. Control animals received the vehicle alone on alternate days and were left to complete the gestation period. In group 2 and 3 laprotomy was done on day 8 and the number of implantation sites were observed prior to the administration of Be on the days mentioned above.

In another set of experiments pregnant rats were treated with Be, sc, on day 5 or 7 and glucosamine 6-phosphate synthase activity in the uterus was estimated on days 6 and 8 of pregnancy. The glucosamine 6-phosphate synthase activity was measured as described in Chapter II.
RESULTS

Effect of Be treatment during pre-and post-implantation period on the length of gestation and the litter size is shown in Table 1. The control rats had an average number of 8.5 implantation sites when observed on day 8 of laprotomy and delivered normal pups on day 22. Implantation sites were absent in rats which were treated with Be on""day 1, 3 and 5 of pregnancy. Bromocryptine injection on days 9, 11, 13 and 15 or on days 14, 16, 18 and 20, 'respectively did not produce any disturbance in the length of the gestation period. Further, these rats delivered on day 22 and the litter size and weights of the pups were comparable to those of control (Table 1).

Treatment with Be on day 5 of pregnancy significantly inhibited the glucosamine 6-phosphate synthase activity in the uterus. However, there was no change in the uterine glucosamine 6-phosphate synthase activity when Be was injected on day 7 (Table 2).

DISCUSSION

Treatment with Be from day 1-5 of pregnancy prevented implantation of the blastocyst as no implantation
sites were observed on day 8 when laprotomy was done. However, treatment from days 9-15 or from day 14-20 did not produce any effect on the embryos and length of the gestation period. All animals delivered normally on day 22 and the weights of the pups and the litter size were comparable with those of the controls.

Blockade of prl release on or before but not after day 7 is known to terminate pregnancy (Sheleshyak, 1955; Kisch and Sheleshyak, 1968; Clemens et al, 1969; Rezabak et al, 1969; Babu and Vijayan, 1983). During early luteinization period Prl stimulates corpus leuteum to develop an increased binding capacity for LH or hCG and to produce P (Holt et al., 1976; Richards and Midgley, 1976). Accordingly, blockade of the hypophysial Prl secretion by Be results in a decrease in ovarian LH/hCG, binding capacity and serum P concentration in rat and man (Holt et al, 1976; Schulz et al, 1976). Progesterone is an absolute pre-requisite for gestation and maintenance of pregnancy and this study has shown that deprivation/inhibition of Prl during early luteinization process by Be led to failure of implantation and thereby pregnancy.

Kelly et al (1976) have shown that a luteotrophin is produced from rat placenta during the second half of the pregnancy which maintains the secretion of progesterone
during the latter part of the gestation period. This explains the observation in this study where Be injection from day 9-15 or day 14-20 did not affect the length of the gestation period, litter size and weight of the pups. The findings of Gorospe and Freeman (1985) also strongly indicate that the developing placenta secretes a Prl inhibitory factor which can directly suppress, via a blood bourne route, the release of Prl at the level of the anterior pituitary gland. Hence the pituitary Prl levels are low during the second half of pregnancy and Be which is a DA receptor agonist directly inhibits the release of Prl from the anterior pituitary gland (Nagasawa et al.,1973; Macleod and Lehmeyer, 1974; Fluckiger et al, 1976) has no effect on the rPL which maintains the second half of pregnancy.

Glucosamine 6-phosphate synthase activity was reduced significantly when Be was injected on day 5. It has been shown that the enzyme activity is high in the endometrium and steroid treatment increases the levels of this enzyme which indicates that the enzyme is localized predominantly in the endometrium of the uterus (Rukmini, 1981). It has also been observed by Tsuiki and Miyagi (1977) that other proliferative tissues like hepatomas show increased levels of this enzyme. The decrease in the enzyme levels in this study may be due to insufficient
secretory transformation of the endometrium caused by low levels of serum progesterone which could have been brought about by the blockade of Prl with Be. However, treatment with Be on day 7 did not cause resorption of the implanted embryos nor any change in the enzyme levels. Rezabek et al (1969) observed termination of pregnancy when an ergoline like compound was injected on day 7. This compound synthesized in their Laboratory may be more potent than Be used in the present study. Another explanation could be that the inhibition of Prl after implantation does not affect the embryo because the production of P in luteal cells after implantation becomes strongly dependent on LH and LH/hCG receptors (Morshige and Rothchild 1974; Rothchild et al, 1974; Akaka et al, 1977). Since Be by itself does not have a teratogenic effect (Muller, 1982) it did not act directly on the implanted embryos and inhibiting Prl levels after implantation as early as on day 7 did not lead to the resorption of the implanted embryos.
Table - 1.
Effect of Bc on the length of gestation and the litter size.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment on day of pregnancy</th>
<th>Mean Day of Parturition</th>
<th>Mean No. of Implantation sites</th>
<th>Mean No. of young Per litter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Nil (4)</td>
<td>22.25</td>
<td>8.5</td>
<td>8.5</td>
</tr>
<tr>
<td>2.</td>
<td>1 to 5 (8)</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>3.</td>
<td>9 to 15 (6)</td>
<td>22.60</td>
<td>8.8</td>
<td>8.8</td>
</tr>
<tr>
<td>4.</td>
<td>14 to 20 (6)</td>
<td>22.70</td>
<td>9.2</td>
<td>9.2</td>
</tr>
</tbody>
</table>

Laprotomy was done on day 8 of pregnancy to record the number of implantation sites in groups 1, 3 and 4. Rats were kept in individual cages for observation till term.
Table - 2.

Effect of Bc on the glucosamine 6-phosphate synthase activity in the uterus of pregnant rats.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment on day</th>
<th>Enzyme activity (n mols/mg protein/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control - D5</td>
<td>52.73 ± 1.15 (5)</td>
</tr>
<tr>
<td>2.</td>
<td>Bc - D5</td>
<td>36.30 ± 2.82* (4)</td>
</tr>
<tr>
<td>3.</td>
<td>Control - D7</td>
<td>61.21 ± 3.16 (4)</td>
</tr>
<tr>
<td>4.</td>
<td>Bc - D7</td>
<td>68.34 ± 1.99 (5)</td>
</tr>
</tbody>
</table>

Bromocryptine was injected on day 5 (group 2) and Day 7 (group 4) and killed 24 hrs after the injection. Controls (group 1 and 3) received equal volume of vehicle alone and were killed 24 hrs later. The values represent mean ± SEM. The number in the parenthesis indicates the number of determinations.

*P<0.001. Vs group 1.