CHAPTER – 3

EFFECT OF PROGESTERONE ON PREGNANCY
Reproductive capacity is fundamentally important, both to individuals and to the health of future generations. Reproductive activity of female includes the ovarian growth, follicular proliferation, ovarian maturation, ovulation and steroidogenesis, which depend upon the periodic release of gonadotropins. The mammalian ovary function includes production of oocytes and essential hormones necessary for the development and function of the female reproductive system. Successful reproduction in the female entails the highly coordinated and synchronous interactions of a wide array of complex processes, such as gametogenesis, sperm–ovum interaction, implantation, embryo development and parturition. The female reproductive cycle is regulated by interaction of the peptide and steroid hormones originating in the hypothalamus, anterior pituitary, adrenal and ovaries (D'Angostino et al., 1989). Any imbalance of levels of hormones results in abnormalities in the female reproductive cycle. Sexual function and fertility are complex reproductive functions that can also be affected by changes in levels of hormones.

The mammalian reproductive system presents a multiple target site for xenoestrogens (Witorsch, 1995). Reproductive toxicity is defined as a dysfunction in the reproductive processes of an organism induced by chemicals. The physiological events associated with fertilization and pregnancy in mammals, including humans, of which relatively little is known about xenobiotics that disrupt these mechanisms by which they mediate these effects are poorly understood (Matt and Brozelleca, 1995; Bhatt, 2000).

A variety of xenoestrogens have been reported to disrupt female reproductive function in laboratory animal and humans; the effects include the disruption of normal sexual differentiation, ovarian function, fertilization, implantation and pregnancy (Khan 1981; Fish, 1996; Soratur and Kaliwal, 1998). In utero exposure to diethylstilbestrol (DES- an antiabortive drug) resulted in reduced fertility, reproductive tract anomalies and increased incidence of vaginal adenocarcinoma in women (Newbold and Mc Lachlan, 1996; Goldberg and Falcone, 1999; Propst and Hill, 2001).

Detection of pre-implantation embryo loss is important for early identification of reproductive failure. It is widely accepted that the developing embryo is particularly sensitive to altered hormone levels during certain periods when sensitive organ systems or types of cells are at risk (Selevan et al., 2000). A particular substance may be without effect on implantation, it may induce gross morphologic defects during early stages of
embryogenesis, and during the fetal period it may have marked effects on the neurologic development. Therefore, investigation of the potential deleterious effects of a substance during pregnancy requires an exposure throughout the entire period of pregnancy (Matt and Borzelleca, 1995). Gonadal steroids act as reversible regulatory agents in the adult; they function as organizational agents during fetal development. Inappropriate exposure of the developing fetus to exogenous estrogens can cause long term deleterious effects. Exposure of toxicants is of particular concern because many feed back mechanisms functioning in the adult are absent and adverse effects may be noted at doses lower than those observed in the adult.

Drug abuse is a serious concern during pregnancy. The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously affect the development of offspring. The potential impact of xenoestrogens on human health is unknown. The effects will be dependent on levels of xenoestrogen exposure, potency or target cell or organ uptake during critical periods of development (Safe, 1995; Pocar et al., 2003). The deprivation and excessive exposure to estrogen neonatally/prenatally can lead to reproductive disorders, including infertility (Mc Lachlan et al., 1975; Jones, 1980; Toppari et al., 1996; Campagna et al., 2001).

In the present study, the effect of exposure to graded doses (1mg, 3.5mg, 7mg, 15mg, 25mg and 50mg/kg body weight) of progesterone on embryo implantation in mice has been analyzed.

The results indicated that all the mated mice were pregnant and have shown a mean number of 10.83 ± 0.447 implantations on the 18th day of pregnancy (Table 3.1). The uterus too disclosed normal implantation sites. No resorptions were observed in control mice indicating no pre-implantation loss (Fig.3.1). Treatment with 1.0 mg and 3.5 mg progesterone/kg body weight causes no loss of implantations. All the mated mice show a mean number of 10.50 ± 0.341 and 10.66 ± 0.421 implantation sites, respectively on the 18th day of pregnancy. There was no significant change in the number of implantation sites when compared to that of the control mice (Fig.3.2 and 3.3). Treatment with 7mg and 15mg progesterone/kg body weight causes a significant decrease in the number of implantation sites (8.83 ± 0.307 and 7.16 ± 0.307, respectively) when compared to that of the control mice (Fig.3.4 and Fig.3.5). No implantations were found
in mice injected with 25mg and 50mg progesterone/kg body weight (Fig.3.6 and Fig.3.7).

The results of the present study indicate that the treatment with 1mg and 3.5 mg progesterone causes no apparent changes in female reproduction. However, treatment with 7mg and 15mg progesterone causes significant ($p<0.001$) inhibition of implantations. Whereas treatment with 25mg and 50mg progesterone causes complete inhibition of implantations.

Pregnancy is a dynamic process with immense anatomic and physiological changes that occur from fertilization to parturition. It is a composite of integrated process where efforts of all endocrine glands mediate through the hypothalamo - hypophysial - ovarian and placental axis (Stock and Metcalf, 1994). Any given agent may have vastly different effects depending on the stage of embryo and fetal development (Matt and Borzelleca, 1995). Implantation of blastocyst in the uterine endometrium is the basic feature of mammalian reproduction. It is the result of a complex series of interactive steps beginning with formation of blastocyst in the uterus and ending with the formation of a definitive placenta (Weitlauf, 1994).

Successful implantation of the blastocyst takes place when uterine endometrium is receptive and capable of implantating the blastocyst, this period is called “implantation window”. In the development of a receptive uterus, estrogen and progesterone play a key role in synchronizing the oviductal transport of the pre-implantation embryo (Finn, 1977). The reduced implantations in the progesterone injected mice may be due to imbalance in estrogen and progesterone rates which are essential for normal implantation (Morries et al., 1967). The absence of estrogen in pregnant mice at the time of implantation induces a state of dormancy of the embryo and implantation is delayed. In order that the uterus becomes receptive, it must first be exposed to progesterone for 48h followed by estrogen (Psychoyos, 1973). Any imbalance in estrogen and progesterone ratio results in adequate uterine decidualization and receptivity (Matt and Borzelleca, 1995).

During gestation, the placenta itself assumes responsibility to produce hormones of gestation. Anything interfering their secretion will result in the termination of gestation (Dickman and Hart, 1972, Glasser et al., 1972, Raziano et al., 1972). The inhibition of implantation by chemicals and hormones may be due to imbalance in the estrogen and
progesterone ratio in mice (Yochim and Zarrow, 1960; Nutting and Meyer, 1963; Prasad et al., 1965). Excess amount of exogenous hormones may cause hormonal imbalance, particularly in the estrogen and progesterone ratio, which is very critical for implantation (Nutting and Meyer, 1963).

It has been reported that the administration of diethylstilbesterol (DES) induces reproductive tract abnormalities (McKinnell et al., 2001). DES has been used as a test chemical in different animal species and its adverse effects on a variety of reproductive endpoints are well characterized (McLachlan and Newbold, 1987; Zimmermann et al., 1991; Liaw et al., 1998). A dose dependant reduction in fertility has been observed in mice exposed to graded doses of diethylstilbestrol from gestational day 9 through 16 (McLachlan et al., 1982). Recent studies reported that the treatment of 17β-estradiol with different combination of xenoestrogens inhibited implantations in rats (Jadaramkunti and kaliwal, 2001) and in mice (Hiremath and kaliwal, 2002; Amstislasvky et al., 2003).

It has been reported that gestational exposure of rats to hydroxyprogesterone affects implantation, weight of embryos and litter size (Pushpalatha, 2004). Piotrowski (1968 a,b) studied the adverse developmental effects of progesterone exposed during embryonic development on chicks, rabbits and rats, where there was intrauterine death, reduction in birth weights, increased resorptions and fetuses with developmental defects. Two progesterone derivatives like medroxy-progesterone and megestral also exhibited adverse effects on ovulation and implantation (Kawashima et al., 1977, 1978). Check et al., (1986) examined the risk of fetal anomalies as a result of progesterone therapy. Rock et al., 1957 reported the antiovulatory effect of the fetal malformations of the synthetic progestins in humans.

The present study has revealed that the treatment with 1mg and 3.5mg progesterone caused no significant change in implantations. Treatment with 7mg and 15mg progesterone caused significant inhibition of implantations. Whereas, treatment with 25mg and 50mg progesterone resulted in no implantations in mice. Inhibition of implantation by progesterone may be due to one or more mechanisms. As has been suggested possible mechanisms by which a reproductive toxicant may produce early pregnancy loss including genotoxic effects on the gametes, alteration in the genital tract, transport of early conceptus, impaired endometrial receptivity to the implanting
blastocyst and post implantation abnormalities of development, hormone transport or nutrition (Wilcox et al., 1988); the accelerator tubal transport or tube locking, delayed transportation of blastocyst, blastotoxic expulsion of the blastocyst from the uterus, cessation of estrogen surge, anti histamine reaction, toxic uterine fluid or imbalance in estrogen and progesterone ratio. Progesterone narcotic effects may play a role in conceptus death after maternal progesterone administration (Petrelli and Forbes, 1964). The author is fully aware of the conclusions of this study, which are not adequate to state the clear mechanism of action of progesterone on pregnancy. Hence, further investigation is necessary.

The results of the present study clearly indicated that treatment with 7mg and 15mg progesterone causes significant reduction in the number of implantations. For further studies, these two doses (i.e., 7mg and 15mg progesterone/kg body weight) have been selected.
Fig. 3.1: Uterus of control showing normal implantations on 18th day of pregnancy. At autopsy diestrus.
Fig. 3.2: Uterus showing implantations of pregnant mice treated with progesterone (1 mg/kg body weight) on 1\textsuperscript{st}, 3\textsuperscript{rd}, and 7\textsuperscript{th} day of pregnancy. At autopsy diestrus.
Fig.3.3: Uterus showing implantations of pregnant mice treated with progesterone (3.5mg/kg body weight) on 1st, 3rd, and 7th day of pregnancy. At autopsy diestrus.
Fig. 3.4: Uterus showing implantations of pregnant mice treated with progesterone (7 mg/kg body weight) on 1st, 3rd, and 7th day of pregnancy. At autopsy diestrus.
Fig. 3.5: Uterus showing implantations of pregnant mice treated with progesterone (15mg/kg body weight) on 1st, 3rd, and 7th day of pregnancy. At autopsy diestrus.
Fig. 3.6: Uterus showing no implantations of pregnant mice treated with progesterone (25mg/kg body weight) on 1\textsuperscript{st}, 3\textsuperscript{rd}, and 7\textsuperscript{th} day of pregnancy. At autopsy diestrus.
Fig. 3.7: Uterus showing no implantations of pregnant mice treated with progesterone (50 mg/kg body weight) on 1\textsuperscript{st}, 3\textsuperscript{rd}, and 7\textsuperscript{th} day of pregnancy. At autopsy diestrus.
Table 3.1: Effect of graded doses of progesterone on embryo implantations on 18th day of pregnancy.

<table>
<thead>
<tr>
<th>Group</th>
<th>Implantations on 18th day of pregnancy</th>
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<tbody>
<tr>
<td>Control</td>
<td>10.83 ± 0.477</td>
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<tr>
<td>Progesterone injected (mg/kg body weight)</td>
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<tr>
<td>1</td>
<td>10.50 ± 0.341 (-3.0) p=0.1985</td>
</tr>
<tr>
<td>3.5</td>
<td>10.66 ± 0.421 (-1.5) p=0.5279</td>
</tr>
<tr>
<td>7</td>
<td>8.83 ± 0.307 (-18.4) p&lt;0.0001</td>
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<tr>
<td>15</td>
<td>7.16 ± 0.307 (-33.8) p&lt;0.0001</td>
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<tr>
<td>25</td>
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<tr>
<td>50</td>
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<tr>
<td>F0.05</td>
<td>105.06</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.0001</td>
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</tbody>
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Values are mean ± S.D. of 6 animals.
Values in parentheses are % change from control.