CHAPTER – 5

EFFECT OF TRANSPLACENTAL EXPOSURE TO PROGESTERONE ON F1 GENERATION MALE FERTILITY
Estrogens are considered primarily as female hormones, but estrogens also play an important role in males (Korach et al., 1996; Hess et al., 1997, Luconi et al., 2002). Exposure of the developing male animals to exogenous estrogens, either in utero or neonatal period resulted in a range of abnormalities of reproductive organ development and function (Arai et al., 1983; Khan et al., 1998; Aceitero et al., 1998; Sharpe et al., 1998; Fisher et al., 1998). In recent years, significant fall in average sperm count (Carlson et al., 1992; Sharpe and Sakkebaek, 1993; Swan et al., 1997) with a progressive increase in incidence of testicular cancer (Miller et al., 1993; Swerdlow, 1993; Kavlock et al., 1996; Toppari et al., 1996) were reported in human and wild-life animals.

Recent studies suggest that besides decrease in the sperm count and quality there is an increase in the malformations of the reproductive apparatus (cryptorchidism and hypospadias) (Paulozzi, 1999; Skakkebaek et al., 2001; Anderson et al., 2000). Prenatal and neonatal exposure of male rodents to various concentrations of natural and synthetic estrogens caused irreversible organizational changes (Brown-Grant et al., 1975; Williams-Ashman, 1988; Weidner et al., 1998; Goyal et al., 2003; Fraser et al., 2006; Henley and Korach, 2006) and also induction of gross abnormalities of the reproductive tract (McKinnell et al., 2001).

In the present study, male fertility was assessed in first generation mice exposed in utero to 7mg progesterone/kg body weight and 15mg progesterone/kg body weight. Sperm count, motility and viability, and sperm functional tests were performed in adult mice exposed to supra-normal levels of progesterone during embryonic development.

The average sperm count in cauda epididymal plasma was found to be 52.6 ± 9.61 millions/gm testes in control mice. In utero exposure to 7mg progesterone/kg body weight exposed male mice showed a significant (p<0.0001) depletion (-51.5%) in sperm count than the control mice (Table 5.1). These changes are more pronounced in mice exposed to 15mg progesterone/kg body weight during embryonic development (Table 5.1 and Fig.5.1).

Significant (p<0.0001) decrease in sperm motility (-10.5%) and sperm viability (-33.11%) was observed in mice exposed to 7mg progesterone/kg body weight transplacentally, when compared with the control mice (Table 5.1; Fig.5.1). These decreases were more in 15 mg progesterone/kg body weight exposed mice during their
embryonic development (Table 5.1; Fig.5.1). A significant (p<0.0001) depletion in sperm coiling percentage was observed in mice exposed to 15 mg progesterone/kg body weight during embryonic development (Table 5.1; Fig.5.1). The average coiling in the normal sperm was 60% whereas, the sperm coiling had decreased to -27.2% and -45.18% in mice exposed transplacentally to 7 mg progesterone/kg body weight and 15 mg progesterone/kg body weight exposed mice, respectively (Fig.5.1). The decreased HOS-positive reaction indicates derangement of plasma membrane and also indication of degenerative changes in the sperm.

Spermatogenesis is a complex process whereby primitive stem cells divide to reproduce themselves for stem cell renewal or they devise to produce daughter cells that will later become spermatocytes. A reduction in sperm count, motility and viability in the transplacentally progesterone exposed mice indicate an inhibitory effect of progesterone on spermatogenesis. In support, Setty and Kar, (1967) observed suppression of spermatogenesis in mice exposed to progesterone through dermal route. Rats exposed to hydroxyprogesterone caproate during embryonic development also showed a decrease in epidydimal sperm count, sperm motility and suppressed reproductive potential (Pushpalatha et al., 2004; 2005).

Results of the present study indicate a significant decrease in sperm count and other sperm parameters in mice exposed to progesterone, prenatally. Prenatal and neonatal exposure of male and female rodents to DES, a very potent synthetic estrogen, causes multiple disturbances in the reproductive tract in adults (Marselos and Tomatis, 1993). There are many reports suggesting a decrease in sperm production and/or fertility at maturity following neonatal and/or prenatal exposure to natural and/or synthetic estrogens in mice (McLachlan et al., 1975; Jones, 1980; Thayer et al., 2001; Kojima et al., 2002), in mares (Ball et al., 1992), in rats (Junkmann and Neumann, 1964; Setty and Kar 1967; Tapanainen et al., 1979; Putz et al., 2001 a,b), in rabbits (Ericsson et al., 1964; Orgebin-crist et al., 1975). Medroxy progesterone has been reported to cause abnormalities in males, when exposed maternally (De Souza et al., 2004).

Hemminki et al., (1998) reported that in utero exposure to estrogen and progesterone causes reproductive abnormalities in both male and female off springs. Colborn et al., (1993) pointed out that prenatal or early post natal exposure to endocrine-
disrupting chemicals could result in permanent and irreversible changes in reproductive potential in wildlife and humans. Prenatal and/or neonatal exposure to estrogenic compounds resulted in smaller testis and reduced sperm production in rodents (Sharpe, 1994; Toppari et al., 1996; Broockfor and Blake, 1997).

During 1950s and 1960s, diethylstilbestrol (DES), a synthetic estrogen, was prescribed for women to prevention of preterm birth. Thirty years after its introduction DES was found to cause sexual deformities and sterility in adults whose mothers were prescribed with DES (Gill et al., 1979; White head and Leiter, 1981; Giusti et al., 1995; Wilcox et al., 1995) and for this purpose was banned in early 1970s. Transplacental exposure to DES results in male and female reproductive tract abnormalities (Mc Lachlan et al., 1980; 1982; Newbold et al., 1991; New bold, 1995; Goldberg and Falcone, 1999). Men exposed to progesterone showed similar results (Heller et al., 1959). Odum et al., (2002) used rats as an experimental model and observed similar results.

The present study indicates that progesterone at low dose i.e; 7mg/kg body weight can reduce the male fertility when compared to control mice. Recently, an increasing number of reports demonstrate deleterious effects of xenoestrogens on the development and function of rodent reproductive organs (Colerangle and Roy 1997; Vom saal et al., 1998, Welshons et al., 1999; Gupta, 2000; Sheehan, 2000). Similar results were obtained in male mice when they were exposed to low doses of β-estradiol-3-benzoate with malformations in the reproductive tract (Putz et al., 2001 a, b). Estradiol benzoate at 10 g/day significantly reduced the male fertility in rats (Meistrich et al., 1975; Crissman et al., 2000).

The results also show that exposure to progesterone significantly decreased sperm production in rats. Brady et al., (2003) reported that exposure to progesterone suppresses gonadotropin secretion and this might be responsible for decreasing sperm number and motility. A study in rhesus monkey (Anand kumar et al., 1980), administered with progesterone, estradiol and norethisterone caused an impairment of spermatogenesis and a significant reduction in levels of circulating serum testosterone. Impairment of adult spermatogenesis in neonatally estrogenised rats has been due to reduced gonadotropin secretion (Brown-Grant et al., 1975; Bellido et al., 1990).
In utero DES exposed sons exhibit several structural and functional abnormalities of the genital tract (Gill et al., 1979). Transplacental exposure of male fetuses to DES resulted in increased cryptorchidism and depleted sperm count (Ohyama, 2004). Progesterone, when administered during pregnancy also resulted in hypospadias (Macnab and Zouves, 1991; Manson and Carr, 2003).

Besides decreased sperm counts, the motility, viability and the sperm coiling percentages were also significantly decreased in experimental animals. These changes were greater in rats exposed to higher doses of progesterone. There are very few studies that have examined the estrogen effects on sperm motility. Goyal et al., (2001) observed reduced sperm motility in DES treated rats. Pushpalatha et al., (2004) reported decreased sperm count and motility in rats exposed prenatally to hydroxyprogesterone caproate. Estradiol at 0.1μg/kg/day dose significantly reduced sperm motility (Gill Sharma et al., 2001). Kaneto et al., (1999) reported reduced motility of sperm when exposed to ethynil estradiol.

Bern (1992) reported reproductive abnormalities in adult rats exposed to estrogen during embryonic development. Bern (1992) also observed long-term permanent changes in adult rats, as a result of exposure to estrogen during embryonic development, without apparent birth defects in the neonates. It is well known that androgens are essential for normal spermatogenesis (Sharpe, 1987). Testosterone deprivation is known to impair the reproductive behaviour and fertilizing ability of the male (Orgebin-Crist et al., 1975). The changes in sperm parameters in the present study may be due to decrease in titres of testosterone in serum of adult mice exposed to progesterone during embryonic development (Chapter-7).

Results obtained in the present study indicate that exposure to progesterone transplacentally caused a significant decrease in sperm quality and quantity; in turn it may affect male fertility. The data also suggests that prenatal exposure to supra-normal levels of progesterone can affect the reproductive potential of male offspring.
Table 5.1: Effect of transplacental exposure to progesterone on sperm count, sperm motility, sperm viability and HOS coiling in 50 day old mice.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>7 mg/kg body weight</th>
<th>15 mg/kg body weight</th>
<th>F_{2,15} ,  p&lt;0.0001</th>
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<tbody>
<tr>
<td>Sperm count (millions/ml)</td>
<td>52.6 ± 9.61</td>
<td>25.49 *± 3.20</td>
<td>17.18 *± 4.70</td>
<td>\text{(-51.5)}</td>
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<td></td>
<td></td>
<td>(-51.5)</td>
<td>(-67.3)</td>
<td></td>
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<tr>
<td>Motile sperm (%)</td>
<td>60.65 ± 10.30</td>
<td>54.70 *± 3.09</td>
<td>33.60 *± 7.33</td>
<td>\text{(-50.5)}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-50.5)</td>
<td>(-46.18)</td>
<td></td>
</tr>
<tr>
<td>HOS Coiled sperm (%)</td>
<td>60 ± 2.82</td>
<td>43.68 *± 8.82</td>
<td>32.89 *± 7.23</td>
<td>\text{(-27.2)}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-27.2)</td>
<td>(-45.18)</td>
<td></td>
</tr>
<tr>
<td>Viable Sperm (%)</td>
<td>67.7 ± 6.84</td>
<td>45.28 *± 5.11</td>
<td>34.38 *± 7.27</td>
<td>\text{(-33.11)}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-33.11)</td>
<td>(-49.21)</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± S.D of 6 animals. Values in parentheses are % decrease from control values are significantly different from control at *p<0.0001.
Fig. 5.1: Effect of transplacental exposure to progesterone on (a) sperm count (b) sperm motility (c) sperm viability (d) HOS sperm coiling in 50 day old mice.

(a) Sperm Count

(b) Motile Sperm

(c) Viable Sperm

(d) HOS Sperm Coiling

Values are mean ± S.D. of 6 animals. Values are significantly different from control at *p<0.0001.
Fig. 5.2: Sperm from control mice showing coiling of tail after exposure to hypotonic solution. 100 X.
Fig. 5.3: Sperm from experimental mice (exposed to 15mg progesterone/kg body wt. during embryonic development) exposed to hypo-osmotic solution showing un-coiled tail. 100X.