CHAPTER – 6

EFFECT OF TRANSPLACENTAL EXPOSURE TO PROGESTERONE ON HISTOMETRIC AND HISTOLOGICAL CHANGES
Review of literature on a vast array of therapeutic agents indicates male reproduction was affected in several ways (Newman, 1984; Welshons et al., 1991). Spermatogenesis and steroidogenesis are the major functions of testis. Spermatogenesis is a process which involves the transformation of undifferentiated germ cells into highly differentiated immature spermatozoa (Clermont, 1972). Spermatogenesis mainly depends on the action of testosterone (Sharpe, 1987). The sertoli cells present in the seminiferous tubules play a central role in development of functional testis, and in expression of male phenotype. Anything interfering with sertoli cells enables the formation of seminiferous cord, prevention of germ-cell entry into meiosis and differentiation of function of the Leydig cells (Mackay, 2000; Atanassova et al., 2005).

The fetal Leydig cells secrete high concentrations of androgens, primarily testosterone, the principal circulating androgen (Haywood et al., 2003; Sharpe et al., 2003; Johnston et al., 2004). Testosterone or its metabolites play an important role in maintaining the reproductive tract, secondary sexual characters, including the maintenance of spermatogenesis (Sundaram and Witorsch, 1995; Hutson et al., 1997; Sharpe, 2001; Drummona, 2006). Germ cell development relies on a highly coordinate interaction with the sertoli cell. The number of sertoli cells and germ cells will determine the quantity of sperm production (Parvinen 1982, Orth et al., 1988; Sharpe, 1994; 1999; Sharpe, 2004; Franca et al., 1998).

Estrogens are known to inhibit the male reproductive function and health. Exposure to excess amounts of normal estrogen and estrogen mimics were reported to decrease sperm production (Kalra and Prasad, 1967; Sharpe, 1998). Exposure to estrogen during development may impair male fertility. Administration of estrogen during neonatal period or adulthood also led to impairment of sperm production and maturation (Steinberger and Duckett, 1965; Meistrich et al., 1975). Large amount of literature is available on the adverse effects of fetal/neonatal estrogen exposure on spermatogenesis and sperm output in adult hood in rodents (Brown-Grant et al., 1975; Arai et al., 1983; Bellido et al., 1990; Aceitro et al., 1998; Khan et al., 1998; Sharpe et al., 1998; Fisher et al., 1998; Sonnenschein and Soto, 1998), and in humans (Stillman, 1982; Sharpe and Skakkebaek, 1993, Toppari et al., 1996).
In the present study, it was demonstrated that gestational exposure to progesterone affects the embryo implantations (Chapter-3), and growth and organ weight of embryos (Chapter-4). Progesterone exposure during embryonic development also affects the male fertility in adult mice (Chapter-5).

This chapter deals with the histological alterations in the testis of adult mice exposed to progesterone during embryonic development.

Histological observations of the testes of the control mice consist of seminiferous tubules and inter tubular elements. The seminiferous tubules show normal spermatogenesis with all cell types and well developed interstitial cells. Each seminiferous tubule consists of a tubular wall with the outer most basement membrane. Resting on the basement membrane are the spermatogonia and sertoli cells. Towards the lumen the primary spermatocytes, secondary spermatocytes and spermatids adhere to sertoli cells. Sperms are seen with heads embedded in the sertoli cells and tails lying in the lumen (Fig.6.1).

Histological observations of the testis of the mice treated with 7mg progesterone/kg body weight showed decreased number of spermatocytes, spermatids and sperms in the lumen of seminiferous tubules (Fig.6.2). Histological study of the testis of the mice treated with 15mg progesterone/kg body weight exhibited decrease in the number of spermatogenic cells, formation of giant cells, vacuoles and less number of sperms in the lumen of the seminiferous tubules. Leydig cells are in deformed conditions (Fig.6.3) The degenerative changes include necropsied spermatogenic cells.

The results indicate that in utero exposure of mice to progesterone affects the spermatogenesis which may be due to an imbalance in the androgens which are essential for normal spermatogenesis.

In the present study the effect of progesterone on testis revealed two principal impacts on the male reproductive system of mice, namely, the anti-spermatogenic and anti-androgenic effects. The anti-spermatogenic effect is reflected by the decreased number of spermatocytes, spermatids in the seminiferous tubules. The anti-androgenic action of progesterone is reflected by decrease in the number of sperms in the tubules of the testis.
The testis is a complex organ containing three important cell types - germ cells, Sertoli cells and Leydig cells. It has two well established functions, namely spermatogenesis and steroidogenesis. The anterior pituitary participates in the control of both these functions through the secretion of gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Steinberger and Steinberger, 1975; Sharpe, 1987; Santen, 1995). Luteinizing hormone secreted by anterior pituitary gland, under stimulation from gonadotropic releasing hormone released from the hypothalamus, acts on the Leydig cells within the testis to induce the synthesis and secretion of testosterone which allows normal spermatogenic development. In the testes, testosterone is synthesized almost exclusively within the Leydig cells (Neaves, 1977; Ewing and Zirkin, 1983). A change in the production and release of testosterone can occur either at the site of the Leydig cell or via an effect on the pituitary or hypothalamus inhibiting the release of LH (Martin et al., 1998).

The testis of humans and other mammals are highly susceptible to damage caused by genetic disorders, environmental or occupational exposure to chemicals or by other means. Several reasons for testicular damage have been catalogued by several workers (Jackson and Ericsson, 1970; Lucier et al., 1977), although these listings are by no means complete. Quality and quantity of sperm have been adversely affected due to exposure to certain drugs. There are reports of varying degrees of testicular dysfunction such as oligospermia, azoospermia, degeneration of the germinal epithelium in testicular biopsies and elevated circulatory FSH and LH levels in mammals after exposure to drugs (Potashnik et al., 1978; Whorton et al., 1979).

The primary function of Leydig cell is the synthesis and secretion of testosterone. This process is under the control of Luteinizing hormone secreted from pituitary, which in turn is regulated by Luteinizing hormone releasing hormone produced by hypothalamus. Testosterone plays an important role in maintaining spermatogenesis (Sundarm and Witorsch, 1995).

Estrogens are known to produce anti-androgenic effects in the males causing azoospermia, reduction of circulatory testosterone levels (Verjans et al., 1974; Hunt et al., 1979) and inhibition of testicular steroidogenesis (Oshima et al., 1967). Higher doses of estrogens are known to inhibit the male reproductive function (Kalra and Prasad, 1967;
Samuel et al., 1964, 1967). Besides testis, the other target organs for estrogens include epididymis, vas deferens, seminal vesicle and prostate gland (Van Beurden-Lamers et al., 1974).

In the study of Goyal et al., (2001), male rats exposed to DES showed a substantial decrease in the diameter of the seminiferous tubules, which may be due to the decrease in androgen circulation. The size of the seminiferous tubule depends on the quantity of androgen produced (Segal and Nelson, 1959; Albert, 1961). Pushpalatha et al., (2003b) reported a decrease in the diameter of the seminiferous tubule and reduction in the number of preleptotene primary spermatocytes, pachytene spermatocytes and sertoli cells in rats exposed to hydroxyprogesterone during embryonic development. Change in testicular architecture could also be due to decreased intra testicular concentration of testosterone due to decreased steroidogenesis.

Exposure to progesterone causes inhibition of spermatogenesis. This may be due to an imbalance in the androgens, which are essential for normal spermatogenesis (Greep et al., 1936; Steinberger and Steinberger, 1975; Sharpe, 1987). Suppression of testosterone production and intra testicular testosterone has been reported in rats following estrogen exposure (Barlow et al., 2003). Damage of the seminiferous epithelium and germ cell loss occurred following estrogen treatment (Newbold and McLachlan, 1985; Fisher et al., 1999).

Neonatal estrogen treatment suppresses FSH secretion (Arai et al., 1983; Bellido et al., 1990; Sharpe, 1993). FSH plays an important role in development of sertoli cells and in spermatogenesis. It is clear that the suppression of FSH will result in fewer sertoli cells (Van Den Dungen et al., 1990; Sharpe, 1994; Sharpe et al., 2003). The decreased sertoli cells may affect the size of the seminiferous tubule and thereby the spermatogenesis cycle.

Transplacental exposure to exogenous estrogens also reported to cause histological changes in fetal/neonatal testis differentiation (Parks et al., 2000, Mylchreast et al., 2002; Fisher et al., 2003). There is substantial literature showing adverse effects of fetal/neonatal estrogen exposure on spermatogenesis and sperm output in adult rodents (Brown-Grant et al., 1975; Arai et al., 1983; Bellido et al., 1990; Aceitero et al., 1998; Fisher et al., 1998; Khan et al., 1998; Sharpe et al., 1998). It was also reported that the
neonatal estrogen exposure results in defects in germ cell development, in adult rats (Sharpe et al., 1998; Atanassova et al., 2000). It is hypothesized that this is due to permanent defects in sertoli cells function, rather than an effect on germ cells themselves (Li and Hindel, 1998).

The present observations were in consonance with earlier reports on prenatal and neonatal administration of estrogen, which leads to mal-development of the testis in rodents (Newbold and Mc Lachlan, 1985; Aceitero et al., 1998; Fisher et al., 1998, 1999; Pushpalatha et al., 2003b). It was observed that the serum testosterone levels decreased significantly (Chapter-7) in mice exposed to progesterone during embryonic development. Hence, it is suggested that exposure to progesterone during early stages of development affects the process of spermatogenesis, which may be through the deprived levels of androgens.
Fig. 6.1: Transverse section of the testis of the control mice showing the presence of normal tubular structure with spermatogenic cells at different stages of development. Seminiferous tubules are packed closely. The tubular spaces are packed with interstitial tissue, containing clusters of Leydig cells. Scale line = 65 µm.

Fig. 6.2: Transverse section of the testis of the mice exposed transplacentally to 7 mg progesterone/kg body weight showing symptoms of arrest of spermatogenesis. The seminiferous tubules are closely packed. The spermatogonia, spermatocytes and spermatids are loosely arranged and less in number. Leydig cells are highly compact and in deformed condition. Scale line = 36 μm.

Fig. 6.3: Transverse section of the testis of the mice exposed to 15mg progesterone/kg body weight during embryonic development showing arrest of spermatogenesis with ruptured epithelium and very few spermatogonia, spermatocytes and spermatids. Scale line = 41.96 μm