SUMMARY & CONCLUSIONS
Reproduction is one of the most important physiological events, that is regulated by endocrine, paracrine and autocrine mechanisms. The anterior pituitary participates in the control of reproduction through the secretion of gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Steinberger and Steinberger, 1975; Sharpe, 1987). The secretion of these hormones are under the control of gonadotropic releasing hormone produced by the hypothalamus. The gonadotropins (FSH and LH) are responsible for the synthesis and secretion of testosterone by the Leydig cells (Ewing and Zirkin, 1983). A block 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 by inhibiting the release of gonadotropins (Martin et al., 1998).

The testes of mammals are highly susceptible to damage caused by genetic disorders, environmental or occupational exposure to xenobiotics or by other means. Specific causes of testicular damage have been catalogued by several workers (Jackson and Ericsson, 1970), although these listings are by no means complete. Quality of sperm production has been adversely affected in the recent years due to the exposure to certain drugs and chemicals. There are reports of increased testicular dysfunction such as oligospermia, azoospermia etc. (Potashnik et al., 1978; Whorton et al., 1979).

Estrogens also have antiandrogenic properties in the males causing azoospermia and reduction of circulating testosterone levels (Hunt et al., 1979), another mechanism of action of estrogenic substances is inhibition of testicular steroidogenesis (Samuel et al., 1964; 1967, Oshima et al., 1967). In certain cases, higher doses of estrogens are reported to inhibit the male reproductive function (Kalra and Prasad, 1967; Samuel et al., 1964; 1967). The target organs for estrogens of any origin in the male are the testis, epididymis, seminal vesicle and prostate gland (Stupf et al., 1971, Van Beurdan –Lamers et al., 1974).

The reproductive system of the mammal is exquisitely sensitive to the synthetic drugs. Recently concern has been expressed over the possibility that exposure to excess female hormone or the estrogenic chemicals may adversely affect male reproduction in wild life and humans (Korach et al., 1996; Hess et al., 1997; Luconi et al., 2002). There is evidence of a significant fall in average sperm count (Carlsen et al., 1992) with a progressive increase in incidence of testicular cancer (Kavlock et al., 1996) in men, for
the past 50 years. Data also suggest increased incidences of certain human male reproductive tract abnormalities such as cryptorchidism and hypospadias during the same period (Toppari et al., 1996).

Neonatal exposure of male rats to diethylstilbestrol resulted in suppression of androgen action and also induction of gross abnormalities of the reproductive tract of adults (Mc Kinnell et al., 2001). It has been hypothesized that all the above male reproductive abnormalities are inter-related and that they may have a common origin in embryonic and/or neonatal life.

Diethylstilbestrol (DES), a synthetic estrogen that was used by physicians to prevent spontaneous abortions in women for more than 25 years, and its use was banned in early 1970s because of its transgenerational reproductive abnormalities. Transplacental effects of DES, on the fetuses of both humans and experimental animals have been confirmed (Mc Lachlan et al., 1980; 1982; Newbold et al., 1991; Bern, 1992). Daughters born to mothers, who took DES suffered from increased rates of vaginal adenocarcinoma, various genital tract abnormalities and abnormal pregnancies. Similar results were observed by Odum et al. (2002) using rat as experimental model. Many studies in rodents and humans suggest that inappropriate exposure to estrogens in utero and during the neonatal period impairs reproduction (Stillman, 1982; Khan et al., 1988; Arai et al., 1993; Sharpe and Skakkebaek, 1993; Topppari et al., 1996).

In view of the above findings, the present investigation has been undertaken to known the effect of transplacental exposure to progesterone on male reproduction in mice.

**Effect of graded doses of progesterone on implantation**

Detection of early embryo loss is important for correct identification of reproductive failure. It is widely accepted that the developing embryo is particularly sensitive to altered hormone levels during certain periods of development (Selevan et al., 2000).

In the present study, the out come data of the investigations used to evaluate developmental toxicity at the end of pregnancy are total implantations, weight of live progeny, as well as malformations.
Prenatal exposure to graded doses of progesterone caused a significant effect on pregnancy by decreasing the number of implantations, when compared to the corresponding control group (Table 3.1). The decrease of implantations was in a dose dependent manner. No implantations were observed in mice injected with 25mg or 50mg Progesterone/kg body wt. (Table 3.1).

Successful implantation of the blastocyst takes place when uterine endometrium is ‘receptive’ and capable of implanting the blastocyst. This period is called “implantation window”. During gestation, the placenta itself assumes responsibility to produce hormones. Any interference in the secretion of hormones 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 the imbalance in estrogen and progesterone ratio in mice (Yochim and Zarrow, 1960; Nutting and Mayer, 1963; Prasad et al., 1965).

Excess amount of exogenous hormones may cause hormonal imbalance particularly in estrogen and progesterone ratio, which is very critical for implantation (Nutting and Mayer, 1963). It was established that exposure to higher doses of estrogen during early stages of pregnancy inhibits the implantations in rats (Cummings and Laskey, 1993; Cavieres et al., 2002).

Gestational exposure to progesterone on food intake, body and organ weights

Gestational exposure to progesterone did not affect the body weights and food intake significantly (Fig. 4.3). Mice exposed to progesterone in utero showed a decrease in the weights of the testis, when compared to their corresponding group of control animals (Table 4.1). Several reports also indicated decreased testes and accessory sex organ weights after exposure to estrogenic compounds (Tullner et al., 1962; Reuber, 1980; Colerangle and Roy, 1997; Gupta 2000; Sheehan, 2000). Abate (2000), reported that steroid hormones affect body weight, food consumption and hypophagic effects on the hypothalamus. The weight of the testis is largely dependent on the mass of differentiated spermatogenic cells and it has been used as a measure of spermatogenesis (Schlappack et al., 1988). Since a strong correlation exists between weight of the testis and number of germ cells (Sinha Hikkim et al., 1989), the reduction in the testicular weight indicates possibility of germinal loss in the progesterone exposed mice.
Gestational exposure to progesterone on sperm parameters

Assessment of the male reproduction sperm quality and quantity are considered important parameters.

Sperm motility have been shown in several studies to be a good predictor of human male fertility (Auger et al., 1994). Sperm motility may be affected by estrogens and thereby fertility. Hypo Osmotic swelling test was also routinely used by several scientists to determine the sperm functional ability. In the present study, sperm volume, sperm motility, sperm viability and sperm functional test were performed to assess the quality and functional status of sperm, which play an important role in male fertility determination.

Gestational exposure to progesterone significantly reduced male reproduction. A significant (p<0.0001) decrease in sperm motility, sperm viability, HOS coiling was observed with a decrease in sperm volume in adult mice exposed to progesterone during their embryonic development (Table 5.1).

Recent studies suggest that besides decrease in the sperm count and quality, there is an increase in malformation frequency of the reproductive apparatus (Cryptorchidism and Hypospadias) (Anderson et al., 2000; Skakkebaek et al., 2001).

Gestational exposure to progesterone on testicular architecture

Histological observations of the testis of the control mice showed that seminiferous tubules contain all stages of spermatogenesis and interstitial cells (Fig. 6.1). The different stages of spermatogenesis are spermatogonia attached to the basement membrane of seminiferous tubule, and towards the centre the primary spermatocytes, secondary spermatocytes and spermatids were found in lumen of the seminiferous filled with sperms. The testis of mice exposed to progesterone in the in utero showed a decrease in the number of spermatocytes, spermatids and sperms in the lumen of seminiferous tubules and the interstitial tissue contains clusters of leydig cells (Fig. 6.2 and Fig. 6.3).

Gestational exposure to progesterone on the steroidogenic marker enzymes and on the levels of serum testosterone, follicle stimulating and luteinizing hormone

The activities of 3β-HSD and 17β-HSD decreased (Table 7.1) significantly in the testis of mice exposed to progesterone prenatally, when compared with their
corresponding controls. The decreased steroidogenic enzyme activity indicates decreased steroidogenesis. The decreased steroidogenesis is known to affect spermatogenesis and reproductive activities in male mice.

The levels of serum FSH and LH increased significantly with a decrease in serum testosterone in mice exposed to progesterone, during embryonic development (Table 7.2). The decreased testosterone levels with increased FSH and LH implies decreased steroidogenic ability of the testis in the experimental mice. Alterations in the hormone levels may alter the accessory sex organ weight and impair sexual behaviour (Sharpe, 1994).

The increase in the levels of serum follicle stimulating hormone could be due to the germ cell loss in the spermatogenic compartment or damage of sertoli cells, thereby affecting the feedback regulation of follicle stimulating hormone secretion.

**Gestational exposure to progesterone on reproductive performance of F1 generation male mice**

Transplacentally progesterone exposed F1 generation adult male mice showed a reduction in reproductive performance to sire offspring in a fixed time period. The 7mg and 15mg progesterone/kg body weight treated male mice were allowed to mate with normal females. Number of implantations, number of fetuses, weight of the fetuses and number of live fetuses were discovered significantly reduced, whereas the number of corpora lutea, number of copulation trials, pre-implantation loss and post-implantation loss increased significantly (Table 8.1), when compared with the corresponding control. The reduction in reproductive performance of progesterone treated male mice was dose-dependent. The reduced reproductive performance in the treated groups may be due to reduced testosterone levels.

Evidence exists in rodents that exposure to synthetic estrogens results in reduction of circulatory androgen levels and this might affect male reproductive abnormalities, which include reduced sperm production and reproductive tract abnormalities (Goyal et al., 2001). Decreased sperm motility, viability and function in prenatal progesterone exposed mice might be responsible for decreased male fertility.
Effect of injection of testosterone into mice exposed to progesterone during embryonic development

Testosterone is thought to be the prominent androgen involved in spermatogenesis (Wright and Frankel, 1979). Exogenous testosterone can restore and maintain sperm production (Chen et al., 1994; Mc Lachlan et al., 1994). Administration of Testoviron depot into experimental mice caused a significant increase in testis weight (Table 9.1 and 9.2). A significant increase in the weights of testis, seminal vesicles and prostate gland were observed following administration of testoviron depot. Injection of testoviron significantly increased the steroidogenic marker enzymes 3β-HSD and 17β-HSD activity levels in the testis of progesterone treated mice (Table 9.3 and 9.4). This may result in increased androgen production, which in turn enhances the male reproductive efficiency.

Serum testosterone levels also significantly increased after testoviron administration with a decrease in serum, the follicle stimulating hormone and luteinizing hormone levels (Table 9.5 and 9.6). The decreased FSH and LH levels indicate the restoration of Sertoli cell function by testoviron.

Sperm count, sperm viability, sperm motility, sperm function was also restored in testosterone administered mice, when compared to the control mice (Table 9.7 and 9.8). This in turn indicates the protective effect of testoviron on suppressed male reproduction in these animals. Several xenoestrogens causing male reproductive abnormalities and restoration of them by administering testosterone has been studied by many researchers (Rao et al., 1993; Mc Kinnell et al., 2001; Wolf et al., 2002; Pushpalatha et al., 2004; Udagawa et al., 2006).

In the present study, exposure to progesterone transplacentally affected the male reproduction in many ways, namely, reducing the sperm count, sperm motility and function, decreasing the testicular 3β and 17β-HSD activity levels and reducing the circulatory testosterone levels. This indicates that progesterone administered prenatally affects male reproduction. When testosterone was administered to treated animals, the above reproductive abnormalities were partially restored. This observation clearly indicates that decrease in male reproductive functions caused through female hormones can be restored by administering testosterone.
CONCLUSIONS

The present investigation was aimed to elucidate the effect of transplacental exposure to progesterone on male reproduction in mice. Studies were also extended to analyze the male reproduction after administration of testosterone to mice exposed to progesterone during embryonic development.

1. Exposure to progesterone during embryonic development caused no effect on body weight and food intake in mice.

2. Exposure to progesterone during embryonic development caused a decrease in the testis and accessory sex organ weights such as epididymides, seminal vesicles and prostate gland.

3. Progesterone exposure during embryonic development caused an inhibition of the marker enzymes of the steroidogenesis, such as 3β-HSD and 17β-HSD in the testis.

4. Progesterone exposure during embryonic development decreased serum testosterone levels, with an elevation of serum FSH and LH levels.

5. Progesterone exposure during embryonic development decreased sperm count, sperm motility, sperm viability and sperm function.

6. Progesterone exposure prenatally caused significant reduction in reproductive performance of first generation males.

7. Administration of testosterone into mice exposed to progesterone during embryonic development showed a partial recovery of the suppressed male reproduction.

The above results suggest that exposure to progesterone during embryonic development caused adverse effects on the male reproduction. The suppressed male reproduction in these animals recovered partially after testosterone administration. The author is fully aware that the conclusions of this study, which are mainly based on gravimetric, histologic, enzymatic and hormonal data are not altogether adequate to understand the clear mechanism of the action of progesterone. Hence, further investigation is necessary to support these findings.