Effect of Exogenous Estrogen during Pregnancy on the Development of the Testis of Rats. Histological Considerations and Clinical Implications

Metwally Abd-El-Bary Mnsoor

Anatomy Department, Faculty of Medicine, Zagazig University
salssabeel_co@yahoo.com

Abstract: Background: Testicular dysgenesis syndrome is a result of disruption of embryonal programming and gonadal development during fetal life. In recent years, evidences have accumulated that exposure to environmental components with estrogenic activity causes reproductive disorders in human population. **Objective:** The study was carried out to evaluate the testicular hazards of the neonatal rats resulting from exposure of their pregnant mothers to estrogenic compounds. **Subjects:** Two primary groups, each consisted of 3 adult male and 12 adult female rats were used. After mating, two secondary groups, each consisted of 6 pregnant rats were divided into control group injected with saline and treated one, subcutaneously injected with oestradiol benzoate 100 µg/kg. body weight/day from the 13th day of pregnancy onwards. **Results:** There is marked decrease in seminiferous tubules of the testis. Some tubules are incompletely formed with blood cells in interstitial tissue of the testis. The nuclei of spermatogenic cells are markedly affected beginning from disturbance in their shapes and chromatin distribution, to pyknosis, to complete disappearance. Their cytoplasm was vacuolated. Sertoli cells showed vacuolation of their cytoplasm and the nuclei of Leydig cells are more heterochromatic. **Conclusion**: Estrogenic compounds administered to pregnant mothers produce marked histological hazards in the testes of their neonates and so, estrogenic compounds must be avoided as possible during pregnancy.

[Metwally Abd-El-Bary Mnsoor. **Effect of Exogenous Estrogen during Pregnancy on the Development of the Testis of Rats. Histological Considerations and Clinical Implications.** *J Am Sci* 2013;9(6):245-250]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 28

Key words: Oestradiol, Testis, Histological study, Albino rats.

1. Introduction

Numerous reports have been recently focused on various aspects of adverse trends in male reproductive health such as; low and probably declining semen quality, high and possibly increasing frequencies of undescended testis, hypospadias and rising incidence of testicular cancer[1-4]. Important biological and histological informations about these trends are usually deficient[5]. Also, experimental and epidemiological studies suggest that testicular dysgenesis syndrome is a result of disruption of embryonal programming and gonadal development during fetal life[6]. All male reproductive problems in humans currently of concern in relation to environmental hazards can be experimentally produced in animals by pre-and perinatal exposure to endocrine disrupters with the exception of germ cell cancer, for which, unfortunately there is no suitable animal model yet[7,8]. In recent years, evidences have accumulated that exposure to environmental components with estrogenic activity causes reproductive disorders in human populations[9,10]. Studies conducted over the past 50 years have clearly shown a continual decline in semen quality accompanied by an increase in male reproductive disorders during this period in industrial countries[11]. These changes in male reproductive function vary from subtle changes to perminant alterations, such as feminization or changes in reproductive behaviour[12]. For example; the sperm count decline have been controversial but large-scale prospective studies using standardized methodologies have shown a decline from 170 to 70 million spermatozoa per milliliter between 1940 and 1990 in Europe[13].

2. Material Aand Methods

The study was carried out on the neonates of two primary groups of adult rats. Each group consisted of 3 adult male, and 12 adult female rats. They were obtained from the animal house, Zagazig Faculty of Medicine. The experiment was performed according to the norms of the Ethical committee of Zagazig University. The rats weighing 160 to 190 g at the start of the study. After mating vaginal smears were performed every morning and the observation of a positive smear was considered the gestational day 0. Pregnant rats were isolated and divided into 2 secondary groups, each consisted of 6 pregnant rats. The rats of the first group were given daily subcutaneous injections of oestradiol benzoate 100 µg/kg.body weight from the 13th day of pregnancy onwards (Folone ampoules of Misr Company for pharmaceutical industries)[14]. The rats of the second group were injected subcutaneously with equivalent volume of normal saline from the 13th day of pregnancy onwards. Four hours after delivery, neonates were killed by decapitation and dissected

with great care where testes were collected and immediately processed for light and electron microscopic examination. Specimens for light microscope were fixed in 10% neutral formol saline for 24 hours. They were processed to prepare 5 µm thick paraffin sections for Hematoxylin and Eosin (H & E) stain[15]. Specimens for electron microscope were immediately fixed in 2.5% glutaraldehyde buffered with 0.1 phosphate buffer at pH 7.4 for 2 hours at 4°C. Then they were dehydrated with ascending grades of ethanol and then were put in propylene oxide for 30 minutes at room temperature, impregnated in a mixture of propylene oxide and resin (1:1) for 1 hour, then in a mixture of previous reagents at 48°C for another one hour. The specimens were embedded in EM bed-812 resin in BEEM capsules at 60°C for 24 hours[16]. By using Leica ultracut UCT we obtained ultrathin (1 µm) sections which were stained with uranyl acetate and lead citrate and were examined with JEOL 1200 Ex-II in Electron Microscopic Center, Faculty of Science, Ain Shams University.

3. RESULTS

1- Control neonatal rats:

A- Light microscopy:

The neonatal testis is surrounded by outer more fibrous layer, tunica albuginea and inner more vascular layer, tunica vasculosa. It contains a fair number of seminiferous tubules, each of them is ensheathed by a thin layer of connective tissue called tunica propria. The seminiferous tubules are separated from each other by connective tissue septa and their lining epithelium is formed of more than one layer of spermatogenic cells (Figs. 1, 2).

B- Electron microscopy:

The seminiferous epithelial cells are two types; spermatogenic cells, the greater ones and characterized by rounded or oval euchromatic nuclei and mostly contain nucleoli. Their cytoplasm is rich in mitochondria. The second type of cells; the lesser one are Sertoli cells which are characterized by oval or elongated euchromatic nuclei and cytoplasm rich in mitochondria. The seminiferous epithelium rests on a basal lamina and ensheathed by myoid cells and connective tissue tunica propria (Figs. 3, 4).

Interstitial cells of Leydig (endocrine cells) are characterized by oval euchromatic nuclei that contain nucleoli. Their cytoplasm is more-electron dense than the surrounding cells and rich in mitochondria and secretory granules (Fig. 5).

2- Oestradiol-treated neonatal rats:

A- Light microscopy:

There is marked decrease in seminiferous tubules in the testis especially in the central part. Incompletely formed seminiferous tubules which are not ensheathed by tunica propria are present. Some blood cells are also present in the interstitial connective tissue of the testis (Figs. 6, 7).

B- Electron microscopy:

The nuclei of spermatogenic cells are more affected than their cytoplasm. The nuclei either irregular in shape or irregular with peripheral clumping of heterochromatin or pyknotic and in some spermatogonia, the nuclei may completely disappeared. The cytoplasm of spermatogenic cells usually shows vacuolations and the basal lamina is disturbed and discontinuous (Figs. 8, 9, 10).

The cytoplasm of Sertoli cells shows vacuolations while the nuclei of interstitial cells of Leydig are more heterochromatic and may be shrunken (Figs. 11, 12).

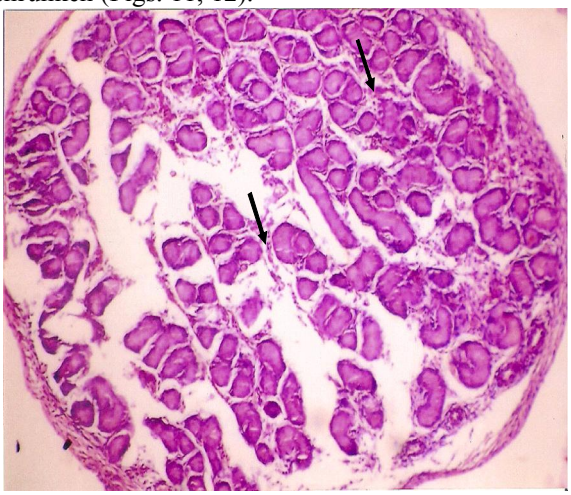

Fig. (1): A photomicrograph of a section in the testis of a control neonatal rat showing fair amount of seminiferous tubules. The tubules are separated by connective tissue septa (arrow). (H & E X 100)

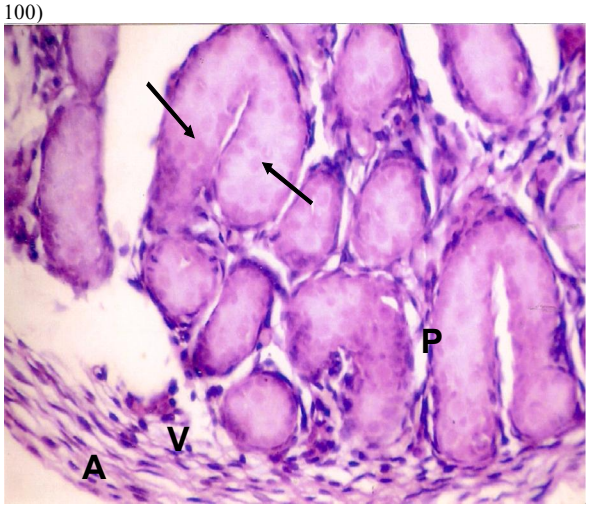

Fig. (2): A photomicrograph of a section in the testis of a control neonatal rat showing tunica albuginea (A) and vasculosa (V) of the testicular capsule. The seminiferous tubules are ensheathed by tunica propria (P) which is a slender connective tissue layer. Seminiferous epithelium (arrow) is formed of more than one layer of spermatogenic cells. (H & E X 400)

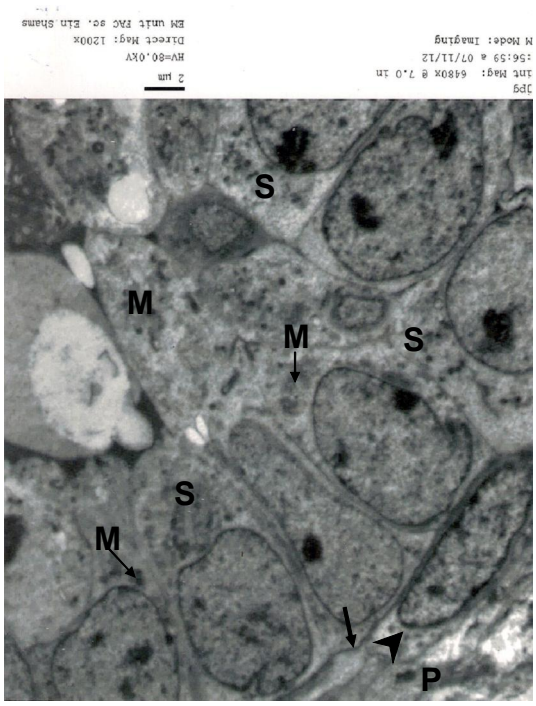

Fig. (3): An electron micrograph of a section in the testis of a control neonatal rat showing the seminiferous epithelial cells; spermatogenic cells (S) with rounded or oval euchromatic nuclei, mostly contain nucleoli and cytoplasm rich in mitochondria (M). The Sertoli cells are less in number with elongated or oval euchromatic nuclei and nucleoli. Their cytoplasm is rich in mitochondria (M). The seminiferous epithelium rests on a basal lamina (arrow) ensheathed by myoid cells (arrow head) and connective tissue tunica propria (P). (X 6480)

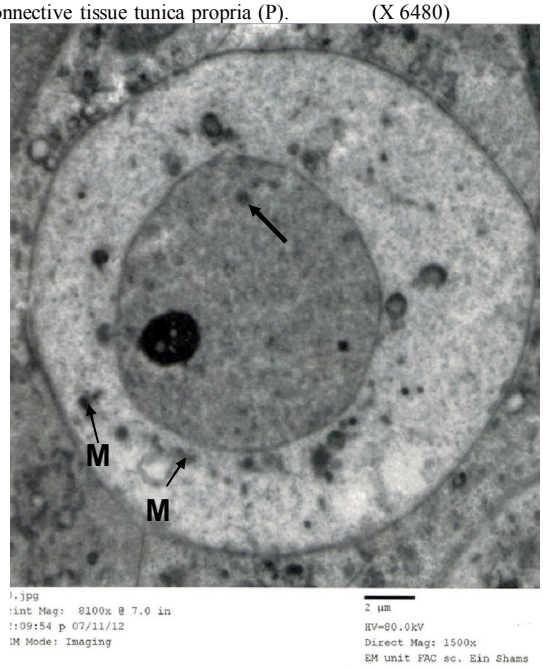

Fig. (4): An electron micrograph of a section in the testis of a control neonatal rat showing higher magnification of a spermatogonium with rounded euchromatic nucleus, nucleolus and minimal chromatin clumping (arrow). The cytoplasm is finely granular and rich in mitochondria (M). (X 8100)

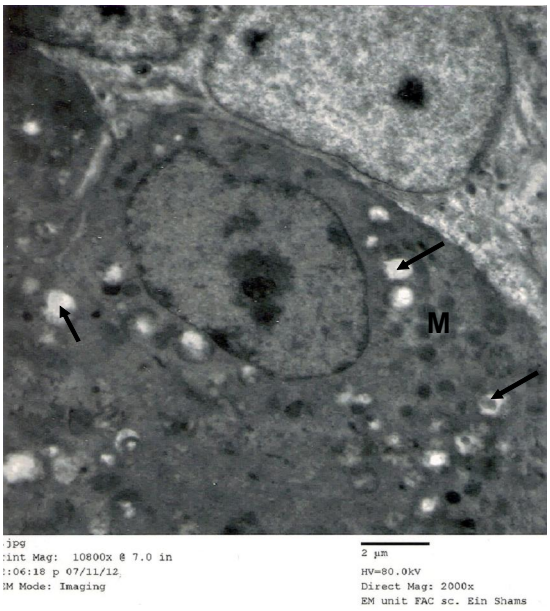

Fig. (5): An electron micrograph of a section in the testis of a control neonatal rat showing Leydig cell; its nucleus is oval, euchromatic and contains nucleolus. The cytoplasm is more electron dense than the surrounding cells and rich in mitochondria (M) and secretory granules (arrow). (X 10800)

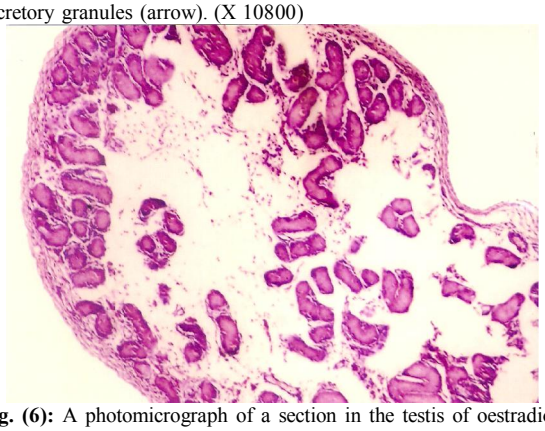

Fig. (6): A photomicrograph of a section in the testis of oestradiol-treated neonatal rat showing marked decrease in seminiferous tubules with more ones towards the testicular capsule. (H & E X 100)

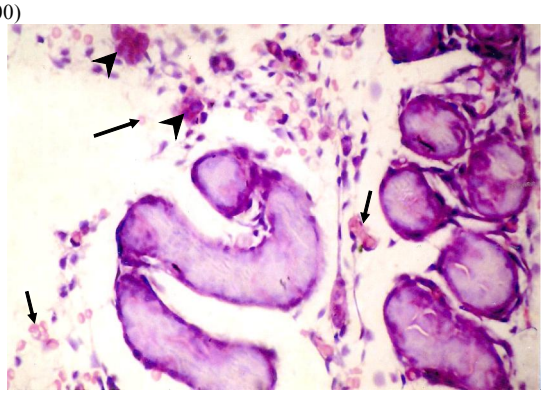

Fig. (7): A photomicrograph of a section in the testis of oestradiol-treated neonatal rat showing some blood cells (arrow) in the interstitial connective tissue. Incompletely formed seminiferous tubules (not ensheathed by tunica propria) are also present (arrow head). (H & E X 400)

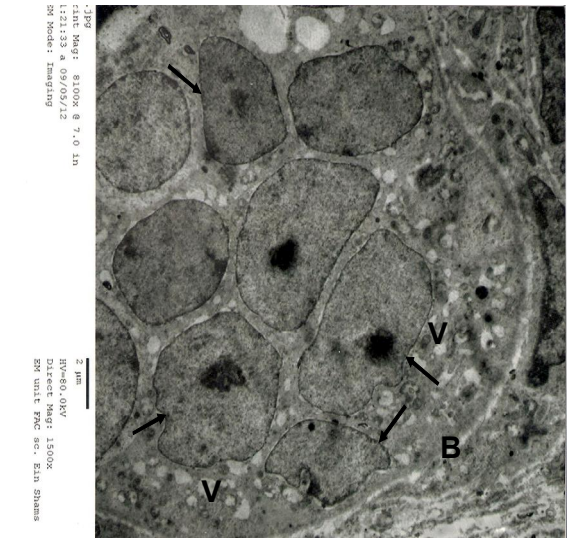

Fig. (8): An electron micrograph of a section in the testis of oestradiol-treated neonatal rat showing spermatogenic cells with vacuolation (V) of their little cytoplasm. The nuclei although still euchromatic but with different irregular shapes and contours (arrow). Basal lamina (B) is disturbed. (X 8100)

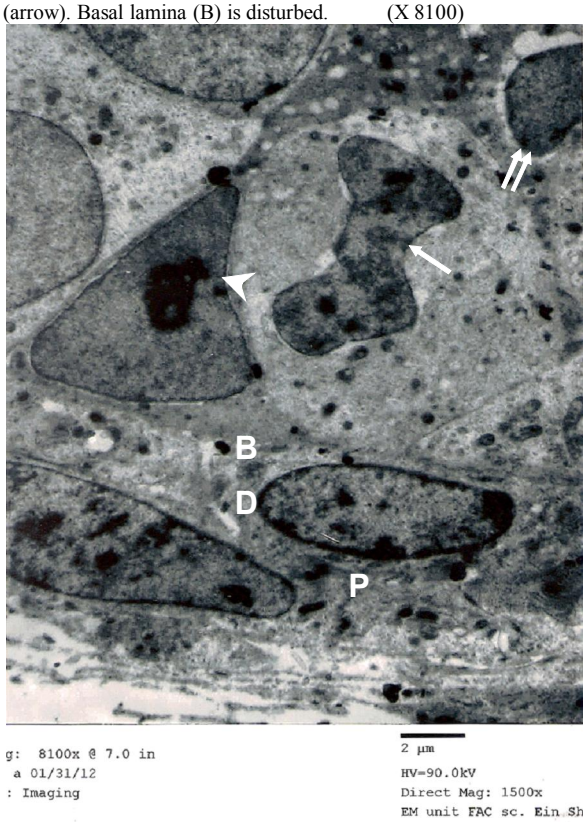

Fig. (9): An electron micrograph of a section in the testis of oestradiol-treated neonatal rat showing spermatogonium with abnormal irregular nucleus and clumping of heterochromatin (arrow). Another one with irregular nucleus (arrow head). A part of pyknotic nucleus (double arrows) of spermatogonium also appears. The basal lamina (B) is disturbed and incontinuous. Myoid cells (D) and tunica propria (P) are nearly intact. (X 8100)

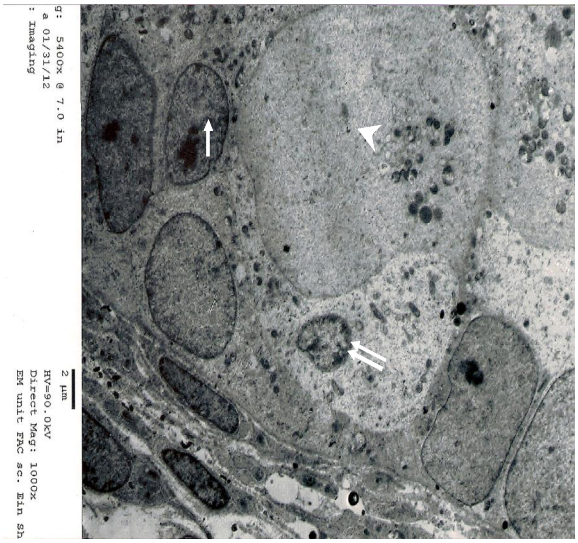

Fig. (10): An electron micrograph of a section in the testis of oestradiol-treated neonatal rat showing spermatogenic cell with heterochromatic nucleus (arrow). Another one with small pyknotic nucleus (double arrows). A third spermatogonium shows complete disappearance of its nucleus (arrow head). (X 5400)

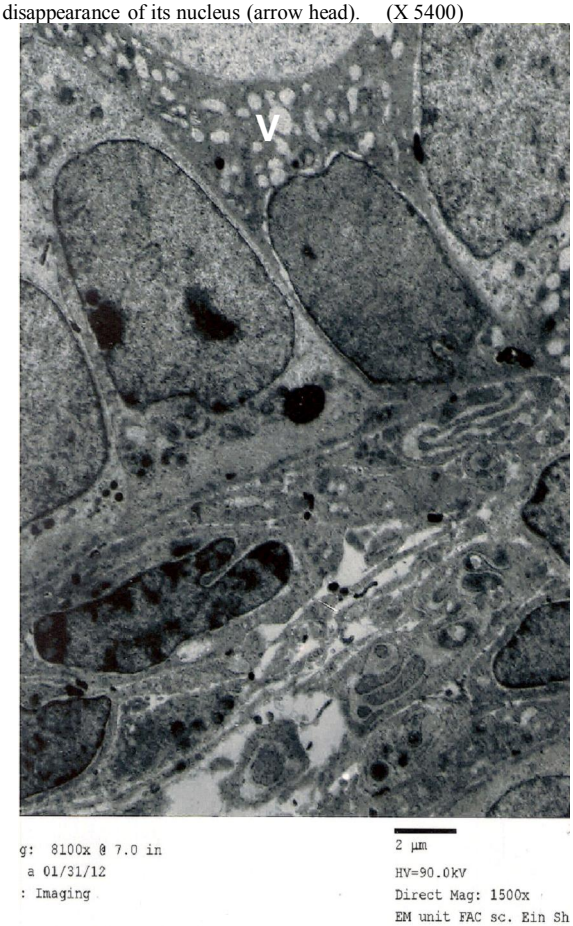

Fig. (11): An electron micrograph of a section in the testis of oestradiol-treated neonatal rat showing Sertoli cell with vacuolation (V) of its cytoplasm. Its nucleus is nearly normal. (X 8100)

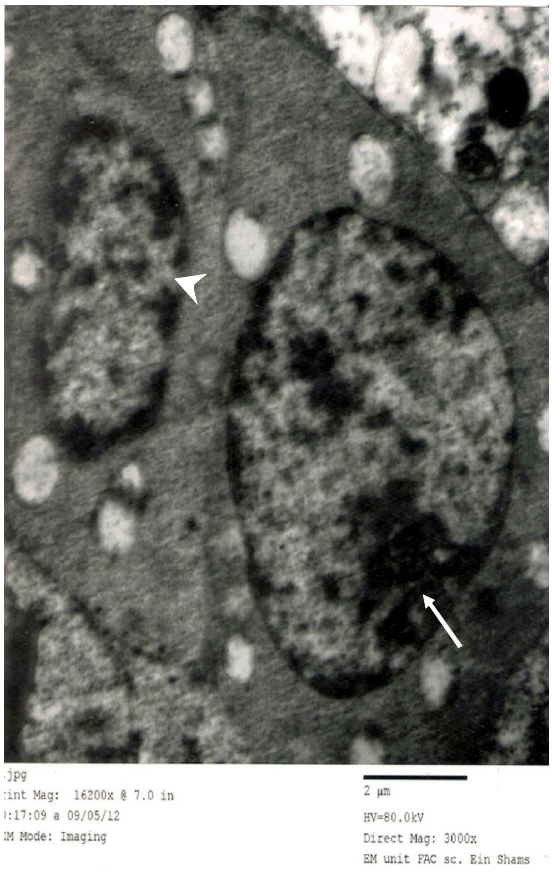

Fig. (12): An electron micrograph of a section in the testis of oestradiol-treated neonatal rat showing Leydig cells with heterochromatic nuclei and peripheral clumping (arrow) of more heterochromatin with decrease in its cytoplasmic mitochondria. One nucleus is shrunken with disturbance of its nuclear membrane (arrow head). (X 16200)

4. Discussion

Due to specialization in medicine, reproductive problems used to be analysed separately by various professional groups as biologists, histologists, paediatric endocrinologists and andrologists[17].

The testes begin to carry out their two major functions; gametogenesis and steroidogenesis during fetal development. Sertoli cells are the first to differentiate, from 13.5 days post conception in rats (42-45 days in human), they surround the germ cells to form the seminiferous tubules[18]. Sertoli cells divide actively until puberty. Spermatogenic cells proliferate until fetal day 17.5 in rats and then they enter a quiescent period untill postnatal day 3. Fetal Leydig cells differentiate soon after Sertoli cells and produce the testosterone and insulin like factor 3 necessary for musculinization of the fetus[19,20].

Oestradiol acts directly on seminiferous tubules and spermatogenic cells. Seminiferous tubules decreased markedly in the testis. The nuclei of spermatogenic cells are markedly affected, starting by changes in their shapes which may proceed to pyknosis and complete disappearance so oestradiol decreases the number and volume of germ cells per testis. Vacuolation of the cytoplasm of spermatogenic cells help in destruction of these cells after disappearance of their nuclei. Decrease in germ cells and seminiferous tubules will lead to low testis weight and volume and decrease in sperm fertilizing ability and consequently sub- or infertility. Affection of Leydig cells may lead to delay in appearance of 2ry sex characters. These results are in accordance with **Storohsnitter et al.**[21] and **Storgaard et al.** [22]. The previous authors added that, some studies have reported alterations in sperm quality and a higher incidence of genital malformation, cryptorchidism and testicular cancer in boys born to women treated during their pregnancy with diethylstilbestrol. The results of the present study are in agreement with **Cupp and Skinner**[23] and **Luconi et al.** [24]. They added that early estrogen treatment may alter seminiferous cord formation and disrupt the development of germ cells, Leydig and Sertoli cells. It also decrease gonocyte number due to decrease in the gonocyte mitotic index and increase in apoptosis.

Two estrogen receptors have been identified; ER1(α) and ER2(β). Both belong to the steroid nuclear receptor superfamily and regulate gene expression. ER1 and ER2 are encoded by two different genes located on different chromosomes (1 and 6 in rats, and 6 and 4 in humans)[25,26]. Numerous human isoforms were described and immunohistochemical analysis has shown that in humans, ERβ is expressed in germ cells, Sertoli and Leydig cells[27]. It is now obvious that estrogenic compounds act directly on spermatogenic, Leydig and Sertoli cells which considered as target cells by their estrogenic receptors.

Estrogenic compounds produce adverse hazards in the testes of male embryos if administered to their pregnant mothers and consequently fertility problems later on. Some researches recorded more severe complications as genital malformation, cryptorchidism and testicular cancer. Unfortunately, all the clinical complications of exogenous estrogenic compounds administered to pregnant mothers are remote complications appear during childhood or adolescence or later on so, exogenous estrogenic compounds must be avoided during pregnancy.

References

1- **Skakkebaek NE, Rajpert DME, Main KM 2001:** Testicular dysgenesis syndrome: an increasing common developmental disorder with environmental aspects. Human Reproduction; 16: 972-978.
2- **Kubota Y, Temelcos C, Bathgate RA, et al. 2002:** The rule of insulin 3, testosterone, Mullerian

inhibiting substance and relaxin in rat gubernacular growth. Molecular Human Reproduction; 8: 900-905.

3- **Toppari J 2002:** Environmental endocrine disrupts and disorders of sexual differentiation. Seminars in Reproductive Medicine; 20: 305-312.

4- **Mackenzie CA, Lockridge A, Keith M 2005:** Declining sex ratio in a first nation community. Environmental Health Perspectives; 113: 1295-1298.

5- **Goyal HO, Robateau A, Braden TD, et al. 2003:** Neonatal estrogen exposure of male rats alters reproductive function at adulthood. Biology of Reproduction; 68: 2081-2091.

6- **Haavisto TE, Adamsson NA, Myllymaki SA, et al. 2003:** Effects of 4-tert-octyl phenol, 4-tertbutyl phenol and diethylstilbestrol on prenatal testosterone surge in the rat. Reproductive Toxicology; 17: 593-605.

7- **Jefferson W, Couse J, Banks E, et al. 2000:** Expression of estrogen receptor b is developmentally regulated in reproductive tissues of male and female mice. Biology of Reproduction; 62: 310-317.

8- **Fielden MR, Halgren RG, Fong CJ, et al. 2002:** Gestational and lactational exposure of male mice to diethylstilbestrol causes long-term effects on the testis, sperm fertility ability in vitro, and testicular gene expression. Endocrinology; 143: 3044-3059.

9- **O'Donnell L, Robertson KM, Jones ME 2001:** Estrogen and spermatogenesis. Endocrine Reviews; 22: 289-318.

10- **Lassurguere J, Livera G, Habert R, et al. 2003:** Time and dose-related effect of oestradiol and diethylstilbestrol on the morphology and function of the fetal rat testis in culture. Toxicological Sciences; 73: 160-169.

11- **Edwards TM, Moore BC, Guillette LJ 2006:** Reproductive dysgenesis in wildwife: a comparative view. International J of Andrology; 29: 109-121.

12- **Anway MD, Cupp AS, Uzumcu M, et al. 2005:** Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science; 308: 1466-1469.

13- **Perez MC, Garcia IM, Ferreras EM, et al. 1996:** Effects of in utero exposure to zeranol or diethylstilbestrol on morphological development of the fetal testis in mice. Journal of Comparative Pathology; 114: 407-418.

14- **Virtanen HE, Rajpert DM, Main KM, et al. 2005:** Testicular dysgenesis syndrome and the development and occurrence of male reproductive disorders. Toxicology and Applied Pharmacology; 207: 501-505.

15- **Bancroft JD, Gamble A 2002:** Theory and practice of histological techniques, 5th ed. Churchill Livingstone, New York, London: 165-175.

16- **Glauret AM, Lewis PR 1998:** Biological specimen preparation for transmission electron microscopy. Volume 17, Portland Press, London.

17- **Skakkebaek NE, Jorgensen N 2005:** testicular dysgenesis and fertility. Andrologia; 37: 217-218.

18- **Olaso R, Habert R 2000:** Genetic and cellular analysis of male germ cell development. Journal of Andrology; 21: 497-511.

19- **Habert R, Lejeune H, Saez JM 2001:** Origin, differentiation and regulation of fetal and adult Leydig cells. Molecular and Cellular Endocrinology; 179: 47-74.

20- **Kaleva M, Virtanen HE, Haavisto AM, et al. 2005:** Circannual rhythm in the incidence of cryptorchidism in Finland-International J of Andrology; 28: 53-57.

21- **Storohsnitter WC, Noller KL, Hoover RN 2001:** Cancer risk in men exposed in utero to diethylstilbestrol. Journal of the National Cancer Institute; 93: 545-551.

22- **Storgaard L, Bonde JP, Olsen J 2006:** Male reproductive disorders in humans and prenatal indicators of estrogen exposure. A review of published epidemiological studies. Reproductive Toxicology; 21: 4-15.

23- **Cupp A, Skinner M 2001:** Actions of the endocrine distruptor methoxychlor and its estrogenic metabolite on *in vitro* embryonic rat seminiferous cord formation and perinatal testis growth. Reproductive Toxicology; 15: 317-326.

24- **Luconi M, Forti G, Baldi E 2002:** Genomic and nongenomic effects of estrogens: molecular mechanisms of action and clinical implications for male reproduction. Journal of Steroid Biochemistry and Molecualr Biology; 80: 369-381.

25- **Hirata S, Shoda T, Kato J, et al. 2003:** Isoform/variant mRNSs for sex steroid hormone receptors in humans. Trends in Endocrinology; 14: 124-129.

26- **Thuillier R, Wang Y, Culty M 2003:** Prenatal exposure to estrogen compounds alters the expression pattern of platelet derived growth factor receptors alpha and beta in neonatal rat testis: identification of gonocytes as targets of estrogen exposure. Biology of Reproduction; 68: 867-880.

27- **Hayley MS, Mason JI, Richard MS 2009:** Steroidogenesis in the fetal testis and its susceptibility to disruption by exogenous compounds. Endocrine Reviews; 30 (7): 2009-2016.

3/2/2013