EFFECT OF ESTRADIOL BENZOATE INJECTION POSTNATALY ON DEVELOPMENT OF THE TESTIS IN ALBINO RAT

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INTRODUCTION AND AIM OF THE WORK

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Estrogen administration to neonatal rats causes impairment of spermatogenesis. This is may be valuable in understanding the mechanisms involved in its effect on the reproductive tract of men whose mothers received estrogen during pregnancy ***(Gill et al., 1979; Newbold et al., 1986 and Sharp and Shakkabake, 1993).***

Prolonged use of estrogen alters the hypothalamic-pituitary-gonadal axis, which in turn, causes persistant inhibition of the spermatogenic process ***(Arai et al., 1983; Bellido et al., 1990 and Pinilla et al., 1992).***

Estrogen also has a direct action on testis which is related to the failure of germ cells to proceed beyond spermatogonia ***(Steinberger and Duckett, 1965)*** and impairment of spermatogenesis ***(Ohta and Takasugi, 1974; Mclachlan et al., 1975 and Ohta, 1977).***

Estrogen induces cryptorchid testes which become exposed to the high temperature in the abdomen. This high temperature prevents the normal maturation of sperms after recovery or administration of gonadotropin ***(Bugnon et al., 1973 and Arai et al., 1983)*** and may cause irreversible effects in some tubule ***(Steinberger, 1971).***

In adult rat exposed neonatally to estrogen there is retention of testicular fluid due to the impairment of spermatogenesis *(Pylkkanen et al., 1991).* This may be secondary to the deleterious effect on growth and structure of the seminiferous tubules and rete testes, of the neonatal estrogenized rodents ***(Orgebin-Crist et al., 1983; Newbold et al., 1986; Greco et al., 1993)*** and men exposed in utero to estrogen ***(Griffin and Wilson, 1994).***

The aim of this study is to know:

1- Is exposure of pregnant female to estrogen leads to abnormalities in the male offspring reproductive system. As the testicular descent in human occurs before birth while in rats after birth. So, to reach this aim we will treat the male rats in the 1st day after birth.

2- The possible mechanisms by which estrogen leads to these abnormalities.

REVIEW OF LITERATURE

ANATOMY OF THE TESTIS

It is the primary reproductive organ in male which is suspended in the scrotum by the dartos muscle and spermatic cord, the left testis usually being about 1 cm lower than the right. Average testicular dimensions are 5 x 2.5 x 3 cm and its weight varies from 10.5-14gm. It is ellipsoidal in shape, compressed laterally and obliquely set in the scrotum ***(Lawrence et al., 1995).***

The testis is enveloped in the tunica vaginalis testis except where the epididymis and the structures within the spermatic cord are attached to the upper pole and posterior surface ***(Basmajian & Slonecker, 1993).*** The right and left sides are separated by the median scrotal septum ***(Sinnatamby, 1999).*** The testis is enclosed by a capsule formed of three distinct layers which from outside inward are:

1- Tunica vaginalis:

It is the lower end of the peritoneal processus vaginalis which is formed before the descent of the testis. After testicular descent, the proximal part of the processus vaginalis, from the internal inguinal ring until the testis contracts and obliterates leaving a closed distal sac in which the testis is invaginated. The tunica is reflected from the testis to the internal surface of the scrotum so, there is:

- Visceral layer which covers all the testis except its posterior aspect and is formed of a single layer of flat mesothelial cells.
- Parietal layer which reaches below the testis and ascends infront and medial to the spermatic cord ***(Lawrence et al., 1995).***

2- Tunica albuginea:

It is a dense bluish white covering composed of interlacing bundles of collagen fibers. It is covered by the tunica vaginalis except at the head and tail of epididymis and posterior aspect of the testis where vessels and nerves enter. Smooth muscle fibers have been observed in the tunica albuginea of the rabbit, man and rat. In rabbit and rat, isolated preparations of testicular capsule may show spontaneous contractions reacting to both cholinergic and adrenergic agents. Abundant autonomic nerve endings near blood vessels have been described ***(Norberg, 1967).*** Non striated myocytes have been demonstrated in the tunica in various rodents and man ***(Holstein, 1967).***

3- Tunica vasculosa:

It is the 3rd inner most layer of the capsule and contains a plexus of blood vessels and a delicate loose connective tissue extending over the internal aspect of the tunica albuginea and covering the septa and therefore all testicular lobules ***(Lawerence et al., 1995).***

Blood supply of the testis:

A- Arterial supply:

It is supplied by the testicular artery from the abdominal aorta which together with the other components of the spermatic cord enter the scrotum. At the upper end of the posterior aspect of the testis, it is subdivided into 2 branches which pass into the medial and lateral surfaces and pierce the tunica albuginea and end in the tunica

vasculosa. From the latter, terminal branches pass into the substance of the testis at various points over the free surface *(Snell, 1995).*

Kormano and Suoranta (1971) noticed that, the intratesticular arteries show coiling of their course.

The capillaries adjoining the seminiferous tubules penetrate the layers of the interstitial tissue which part of the blood testis barrier. They don't enter the walls of the tubules being separated from the germinal and supporting cells by basement membrane and by a variable amount of interstitial tissue containing Leydig cells. At this level, highly selective exchange phenomena occurs involving endogenous and immune substance *(Neaves, 1977).*

B- Testicular veins:

They emerge from the back of the testis and unite, to form extensive venous plexus termed the pampiniform plexus which ascends in the spermatic cord. This plexus is drained by 3-4 veins which enter the abdomen via the deep inguinal ring and unite into 2 veins which become a single vein which open in the inferior vena cava, on the right side and in the left renal vein on the left side *(Sinnatamby, 1999).*

C- Lymph vessels:

They end in the lateral and pre-aortic lymph nodes on the sides of the abdominal aorta, at the level of the first lumbar vertebra *(Snell, 1995).*

Autonomic nerve fibers:

Autonomic terminals are not closely applied to smooth myocytes, but end a variable distance from their surface ***(Richardson, 1962).***

Non myelinated autonomic axons branch into tapering, varicose collaterals. At the zone of transmission there are clusters of vesicles. In sympathetic fibers, these vesicles form a dense-cord and correspond in positions to the characteristic fluorescence of catecholamines seen when preparations fixed in formaline vapour are viewed with ultraviolet light ***(Falck and Owman, 1965).***

Catecholamineferous nerve fibers occur in the human testis and epididymis forming plexuses around smaller blood vessels and among the interstitial cells ***(Baumgarten and Holstein, 1967; Hodson, 1970).***

They are derived from the tenth and eleventh thoracic spinal segments via the renal and aortic sympathetic fibers and are also accompanied by afferent sensory fibers. Such nerve fibers form plexuses around the smaller blood vessels and amongst the inter stitial tissue cells ***(Lawrence et al., 1995).***

NORMAL DEVELOPMENT OF THE TESTIS

The gonads are derived from (1) mesothelium lining the posterior abdominal wall, (2) underlying mesenchyme and (3) the primordial germ cells ***(Moore and Persaud, 1993). Collins (1995)*** added that there is a fourth source which is the invading angiogenic mesenchyme already present in the mesonephron. The primordial germ cells are spherical in shape and larger than the surrounding mesenchymal cells. They have rounded vesicular nuclei and abundant cytoplasm which is less eosinophilic than that of the mesenchymal cells ***(Gondos and Conner, 1973).*** These primordial germ cells are visible early in the fourth week in the wall of the yolk sac. Later on, they migrate along the dorsal mesentery of the hind gut to the gonadal ridge and become incorporated in the primary sex cords ***(Moore and Persaud, 1993).***

Collins (1995) mentioned that the primordial germ cells in male give rise to the precursors of the male germ cells termed primitive spermatogonia (prespermatogonia) which remain quiescent until late adolescence where they begin to undergo mitotic division. The ordinary epithelial cells of the sex cords on the other hand become the constitutive epithelium of the seminiferous tubules and they develop into the Sertoli cells. The primordial germ cells are of large size in comparison with most somatic cells being from 12 to 20 um in diameter and are characterized by vesicular nuclei and well defined nuclear membrane and by the tendency to retain yolk inclusion long after these have disappeared from somatic cells.

In human embryo, the germ cells can be identified in a stage when the number of cells is probably not more than 20-30 ***(Hardisty, 1967).*** When the tail fold has formed, they appear within the endoderm, the splanchnic mesoderm of the hind gut as well as the adjoining region of the wall of the yolk sac. They migrate by amoeboid movement and by growth displacement to reach the medial side of the mesonephric ridges which are the developmental site of the gonads ***(Collins, 1995).***

PRENATAL DEVELOPMENT OF THE TESTIS

In all embryos and at an early stage of gonadal development termed indifferent (Ambisexual) stage, it was thought that the development of one sex phenotype or another occurred after migration of the primordial germ cells to the indifferent gonads. However, development of male or female gonads, genital ducts and external genitalia occurs as a result of a complex interplay between genetic expression and effect of sex hormones *(Collins, 1995).*

1- The indifferent stage:

The formation of gonads is indicated at the fifth week when a thickened area of coelomic epithelium develops on the medial aspect of the mesonephros. Proliferation of these epithelial cells lead to bulge on the medial side of each mesonephros known as gonadal ridge. Finger-like epithelial cords called primary sex cords soon grow from the gonadal ridge into the underlying mesenchyme. The indifferent gonad now consists of an external cortex and an internal medulla *(Moore and Persaud, 1993).* Up to the seventh week of gestation, the indifferent gonad possesses no sexual differentiating features *(Collins, 1995).*

2- Stage of differentiation of sex:

Male embryo has an "XY" chromosome. Although the pattern of sex chromosomes determines the choice between male and female developmental pathway, the subsequent phases of sexual

development are controlled not only by sex chromosomes but also by hormones *(Sadler, 1996).*

Development of the male phenotype requires the y-chromosome. Only the short arms of this chromosome are critical for sex determination. The gene for the testis determining factor (TDF) is localized in the sex determining region of the y-chromosome *(Burgoyne et al., 1988; Palmer & Burgoyne, 1991).* This gene is located near the tip of the short arm of Y-chromosome *(Collins, 1995).*

The Y-chromosome has a strong testis determining effect on the medulla of the indifferent gonad. When it is present, the medulla differentiates into a testis and the cortex regress *(Moore and Persaud, 1993).*

It was believed that the gonads were indifferent until the arrival of the primordial germ cells in the gonadal ridge *(Collins, 1995).*

The primordial germ cells migrate by amoeboid movement along the dorsal mesentery of the hind gut. It reaches the primitive gonads at the beginning of fifth week and invades the genital ridge in the sixth week. If it fails to reach, the gonads will not develop. So, the priomordial germ cells have an inductive influence on the development of the gonads into male or female *(Sadler, 1996).*

3- Advanced prenatal development:

A- Seminiferous tubules:

In embryo with Y-chromosome, the TDF induces the sex cords to condense and branch, their ends anastomose to form the rete testis. The sex cords now called seminiferous cords. They lose their connection with the germinal epithelium so a thick fibrous capsule called tunica albuginea develops. The seminiferous cords develop into the seminiferous tubules, tubuli recti and rete testis. The wall of the seminierous tubules is composed of 2 kind of cells; Sertoli cells (derived from surface epithelium) and spermatogonia (derived from primordial germ cells) ***(Moore and Persaud, 1993).***

The seminiferous tubules of the human do not acquire lumina until the seventh month but the tubules of the rete testis become canalized somewhat earlier ***(Collins, 1995).***

B- Interstitial tissue:

The seminiferous tubules become separated by mesenchyme that gives rise to interstitial cells of Leydig. By about the eighth week, these cells produce testosterone hormone ***(Moore and Persaud, 1993).***

Testicular descent:

The testis at first lies on the dorsal abdominal wall. As it enlarges its cranial end degenerates and the remaining organ therefore occupies more caudal position ***(Collins, 1995).***

By about 28 weeks of gestation in human, the testis has descended from the posterior abdominal wall to the deep inguinal ring. This change in position occurs as the pelvis enlarges and the trunk of the embryo elongates. This descent in the abdomen is largely due to relative movement that results from the growth of the cranial part of the abdomen away from the caudal part (further pelvic region). Few data is known about the cause of the testicular descent through the inguinal canal into the scrotum but the process is controlled by androgen produced by the fetal testes ***(Wensing, 1988).***

Descent of testis through the inguinal canal begins during the 28^{th} week of gestation and takes 2-3 days. About the 32^{nd} week of gestation, the testis enters the scrotum. After it enters the scrotum, the inguinal canal contracts around the superficial ring. More than 97% of full term babies have both testes in their scrotum. During the first three months after birth the most of undescended testes descend in the scrotum ***(Moore and Persaud, 1993).***

The descent of the testis is mediated by the gubernaculum which is contractile under the influence of testosterone. This occurs at some point between the 22^{nd} and the 23^{rd} week after fertilization ***(Mclachlan, 1994).***

As the testis descends, it is accompanied by its peritoneal covering, and the adjoining peritoneum from the iliac fossa is drawn down into the processus vaginalis. The distal end of the processus

vaginalis in the scortum into which the testis projects form the tunica vaginalis testis but the peritoneum associatd with the spermatic cord in the scrotum and in the inguinal canal normally becomes obliterated and forms a fibrous reminant termed the vestigue of the processus vaginalis. In the rat, it has been shown that the gubernaculum is highly contractile during descent of testis *(Collins, 1995).*

Environmental effect on the testicular development:

Disorders of development of the testis and reproductive tract in male fetus are increasing in incidence. Testicular maldescent and hypospadius appear to have doubled or trebled in incidence in the last fifty years *(Giwercman and Skakkabaek, 1992; Sharp and Skakkebaek, 1993).*

It is well established that administration of estrogen to pregnant animals during the period when the testicular differentiation and musculinization are occurring in male fetus will lead to abnormalities in these processes. Also this will lead to increase risks of cryptorchidism and hypospadius at birth and smaller testes with reduced sperm counts in adult life *(Greco et al., 1993).*

The same is true for man based on studies of male offspring of the six million women worldwide who were administrated diethylstilbestrol to prevent miscarriage *(Stillman, 1982).*

Sharp and Shakkebaek (1993) suggested that there has been a generally increased human exposure to estrogen over the last fifty years. This has been caused by the changes in diet and body composition (more fat)leading to increased exposure of women to their own estrogens and increased exposure to environmental estrogens. The most important of which are the range of pollutant chemicals which when ingested mimic the effects of estrogens in the body by interacting with the receptors for estradiol *(Adlercreutz, 1990)*.

POSTNATAL DEVELOPMENT OF TESTIS

There is a quiescent period of 10 to 14 years between birth and onset of testicular growth. In rat, an increase in the testicular weight occur just after birth and continues until the adult size is attained. It is associated with an increase in the tubular diameter and length as well as with onset of spermatogenesis. The latter begins as early as five days after birth ***(Emil and Anna, 1975).***

In human testis, the cell cords develop lumina during childhood around the seventh year of life. The seminiferous tubules appear as long convoluted flattened loops. At the point where each loop closely approaches the mediastinum testis the tubules become connected with the rete testis by straight canaliculi called tubuli recti. Before puberty, the seminiferous tubules are small and composed of two types of cells: (1) Sertoli cells (supportive and nutritive) and (2) Gonocytes (precursors of spermatogonia) ***(Cormack, 1987).***

HISTOLOGY OF TESTIS

A- Lobules of testis:

Clermont and Huckins (1961) and Roosen-Runge (1961) have described the testicular lobules in rats as closed loops, opening at both ends into the tubuli recti and thus into the rete testis and this pattern is now generally accepted. The tubules are supported by loose interstitial tissue formed by Leydig cells containing yellow pigment granules, blood vessels, extensive lymphatics and numerous macrophages. The tubules in each testis are about 400 to 600 in number and 70 to 80 in length. Their diameter varies from 0.12 to 0.3 mm. Seminiferous tubules are pale early in life but in old age they contain much fat and are deep yellow. Each tubule has a basal membrane containing numerous elastic fibers with flat cells between layers and covered externally by flat epithelioid cells. With age, the basement membrane becomes thicker and denser. Internal to it, the seminiferous tubules consist of spermatogenic cells and supporting cells. The number of the lobules in human testis being (200 to 300). They differ in size. The central ones are largest and longest. Each have 1-3 or more convoluted seminiferous tubules *(Lawrence et al., 1995).*

Cormack (1987) mentioned that the human seminiferous tubules before puberty are small and composed of two types of cells; Sertoli cells and gonocytes. During the adolescence (10-14 years of age) the gonocytes (now termed spermatogonia) start to proliferate producing millions of spermatozoa.

B- Sertoli cells:

These are the non germinal element in the complex cellular population of seminiferous tubules, and are variable in shape and nuclear configuration. Changes in form and distribution from birth, to the onset of puberty have shown that there is progressive diminution in number from 3 years onward. But this is due to testicular growth and the number remains constant *(Nistal et al., 1982).*

Before puberty, the Sertoli cells in human testis are numerous and have small irregular nuclei and poorly defined cytoplasm. After puberty, they enlarge and have large pale polymorphic nuclei and abundant cytoplasm. They extend deeply toward the lumen of the seminiferous tubules *(Cormack, 1987).*

Sertoli cells are suggested to utilize the lipids from ingested residual bodies shed by spermatids for hormonal synthesis *(Lacy and Pettit, 1970).* Inside Sertoli cells, there are intracytoplasmic microfilaments and microtubules. These play a role in bringing about the changes in the Sertoli cells that permit the germ cells to ascend the sides of the Sertoli cell in their deep recesses to be released as spermatozoa. The adjacent Sertoli cells are held together by gap junction and multiple tight junctions. The tight junctions are situated near the base of the cells and separate the intervening spermatogonia from the more superficial spermatocytes and spermatids. This arrangement is important to allow the circulating hormones to reach the spermatogonia, the spermatocytes, the

spermatids and the spermatozoa. The latter, lie near the lumen of the tubules and are separated from the tissue fluid by tight junctions between the Sertoli cells, which are referred to as (the blood-testis barrier) because they protect the germ cells from the circulating noxious substances. However, the presence of the germ cells in the recesses, of Sertoli cells, allows nutrients to pass through the plasma membrane and cytoplasm to enter these germ cells *(Cormack, 1987).*

C- Spermatogonia:

They are the stem cells for all spermatozoa and are derived from the primordial germ cells and become gonocytes. In the fully differentiated testis, they appear along the basal lamina of the seminiferous tubules *(Lawrence et al., 1995).*

Before puberty, gonocytes are fewer than Sertoli cells, in human testis and have dark, large spherical central nuclei and clear peripheral cytoplasm. During adoloscence, the gonocytes called spermatogonia start to proliferate producing millions of spermatozoa. The spermatogonia are large rounded cells situated along the basal membranes in between tall Sertoli cells and embedded in their cytoplasm *(Cormack, 1987).*

In man, three types of spermatogonia can be distinguished and are termed dark type A, pale (light) type A and type B. Spermatogonia are large rounded cells in the three types and show little difference in size or in their cytoplasm. But the light and dark types are distinguishable by their nucleoli which are eccentric and

attached to the internal aspect of the nuclear membrane. Type B have more constantly spherical nucleus in which the nucleolus is central in position. The dark type A is differentiated from the pale type A by dark nucleoplasm and large pale staining nuclear vacuole *(Lawrence et al., 1995).*

D- Spermatogenesis:

It begins in rats as early as 5 days after birth. In man quiescent period of 10-14 years occurs between birth and the onset of testicular growth *(Emil and Anna, 1975).*

Normal spermatogenesis can occur only if the testes are at temperature lower than, that of the abdomen. In the scrotum they are at a temperature about 3°C lower than that of abdomen. The control of the testicular temperature is not fully understood but the surface area of the scrotal skin can be changed reflexly by the contraction of the dartos and the cremaster muscles. It is now recognized that testicular veins, in the spermtic cord that form the pampiniform plexus, together with the branches of the testicular artery which lie close to the veins probably assist in stabilizing the temperature of the testis by a countercurrent heat exchange mechanism. By this means, the hot blood arriving to the arteries from the abdomen loses heat in the blood ascending to the abdomen within the veins *(Snell, 1995).*

In the rat, six successive generations of spermatogonia produced by divisions with rather fine morphological differences are termed type A1, A2, A3, A4, intermediate and B. The mitotic

division of the stem cells produces the type A spermatogonia, which in turn by mitosis also gives rise to the cells of the next generation, A1, A2, A3 and A4.

A4 spermatogonia by mitotic division give rise to the next generation (In) intermediate spermatogonia which divide to give the sixth and last generation of spermatogonia which is the type B spermatogonia which divide to preleptotene primary spermatocytes (PL). As the spermatogenic cells proceed through the prophase of the first meiotic division, they pass through a series of morphologically distinct phases; preleptotene (PL), leptotene (L), zygotene (Z), pachytene (P), diplotene (D) and diakinesis. The last phase divides by the first meiotic division and gives rise to the 2ry spermatocyte which quickly completes the second meiotic division and gives rise to the haploid spermatids. The spermatids pass through a series of morphological changes (spermiogenesis) to become sperms *(Michael and Edward, 1985).*

Cormack (1987) described three types of spermatogonia in human. Dark type A (dark stained nucleus), pale type A (pale stained nucleus) and type B (spherical nucleus with chromatin clumps along the nuclear membrane). A spermatogonia divide to give dark type A (to maintain number of spermatogonia) and pale type A which becomes differentiated into type B spermatogonia that divide by mitosis giving 1ry spermatocytes which migrate toward the middle zone of the tubule. The 1ry spermatocytes via the first meiotic division give rise to smaller 2ry spermatocytes having haploid

number of chromosomes, and enter 2nd meiotic division to give small cells (spermatids) which undergo spermiogenesis to form the spermatozoa with the same haploid number of chromosomes. The spermatid has a central rounded nucleus, a well defined Golgi apparatus, numerous mitochondria and two centerioles. Inside the Golgi apparatus, an acrosormal granule appears which together with its surrounding membrane become adherent to the nuclear membrane. The acrosomal membrane with the granule inside spreads around the anterior half of the nucleus as acrosomal cap (Head cap). The nucleus elongates and flattens, the centerioles migrate and acquire a flagellum which become surrounded by the cytoplasmic tubule. The mitochondria form a spiral sheath around the flagellum which has a terminal ring at its end. Both the sheath and the terminal ring become surrounded by the middle piece (body of the sperm). A part of the cytoplasm forms a thin covering around the nucleus, the middle piece and tail piece while the rest becomes detached forming the residual body.

E- Efferent ductules:

The events described above occur in the highly coiled parts of the seminiferous tubules. As these reach the lobular apices they are less convoluted, assume an almost straight course and unite into 20-30 larger but shorter straight ducts about (0.5 mm) in diameter (tubuli recti). The straight seminiferous tubules enter the fibrous tissue of mediastinum testis ascending backwards as a close network (the rete testis) of anastomosing tubes lined by a cuboidal epithelium. At the upper pole of the mediastinum, 12-20 efferent

ductules perforate the tunica albuginea to pass from the testis to epididymis. The efferent ductules are lined by a columnar epithelium most of its cells are ciliated. The lining cells of the efferent ductules are higher than the cuboidal epithelium of the rete testis and lower than the tall columnar epithelial cells of the epidiymal duct *(Lawrence et al., 1995).*

ESTROGENS

Estrogens are a class of steroid hormones linked principally with the control of female sex organ being responsible for reproduction. However, male also produces measurable levels of estrogen. There are 3 forms of estrogens:

- Estradiol (E2): Synthetized by ovary.
- Estriol: Estrogen secreted by placenta.
- Estrone: Present in postmenopausal female (here it is produced by peripheral conversion of androstenedione which secreted by adrenal cortres in adipose tissue by aromatization of androgens).

Only 2% of estradiol is free in blood where the rest is bounded to 6 hormone binding globulin.

(Korach, 1992)

Estrogen is used in:

1- Combination with progesterone to inhibit ovulation as a contraceptive.

2- Replacement therapy after menopause to overcome post-menopausal symptoms.

3- Replacement therapy in hypogonadic conditions for development of 2ry sexual character in case of ovarian deficiency already present before puberty.

4- Dysfunctional uterine bleeding.

5- To suppress postpartum lactation.

6- Palliative treatment for hormone dependent breast cancer.

7- Hormonal treatment of androgen-dependent prostatic cancer. It is used to induce medical castration resulting in suppression of testicular androgen *(Munson, 1995).*

Estrogen during pregnancy and fetal period:

- Slowly increase in 1st four weeks.
- Rapid increase until twenty weeks.
- Slowly decrease until delivery (decrease to normal within 5 days).

Fetal ovaries are not histologically distinguishable until about the 10th- 11th week of gestational age. Although fetal ovaries cannot synthesize steroids denovo at this period, it is probable that small amounts of estrogens are produced by aromatization in the granulosa cells by the 10th week *(George and Wilson, 1978)*. Their exact nature and local significance remain obscure. Moreover, it appears that the female internal genital tract development does not depend on any hormonal or other influences from the ovary *(Sizonenko, 1993)*. The fetus is in a significantly high estrogen environment owing to the high levels of estrogen produced by maternal placenta along with progesterone during this period. This occurs particularly during the last days of pregnancy, where the circulating placental levels of sex-steroid hormones reach adult amounts. However, there are no visible estrogenic effects observed in the neonate besides a rare transient breast enlargement and clitoral enlargement. This is probably due to high circulating levels of α-feto-proteins (α-FP), which bind estrogen and protect the fetous from such high circulating levels of free hormones. The α-FP interact only with endogenous steroidal

estrogens and does not bind synthetic estrogen. Still, there are some differences between steroid secretion during pregnancy and the pattern present in non pregnant female. Estrogen from placenta is quite different from that of the ovaries ***(Diczfalusy, 1964)***.

It is not definite at the present what the optimal levels of maternal estrogens are of normal fetal development but it is well known that the alterations in the maternal estrogen levels during pregnancy can significantly affect both female and male fetuses producing permanent changes in several estrogen-target tissues. In fact, several years ago pregnant females were given diethyl stilbsterol during pregnancy to avoid miscarriage and other potential side effects induced by pregnancy. In the following years, multiple reports described malformations and lesions of estrogen target organs in male offsprings ***(Herbst et al., 1971 and Horwitz et al., 1988)***. Several experimental studies clearly provided evidence for the cause and relationship between abnormal levels of estrogenic compounds during gestation and presence of alterations in several organs and systems in the offspring in later stages of life ***(McLachlan et al., 1980 and Migliaccio et al., 1992).***

MATERIAL AND METHODS

MATERIAL AND METHODS

1- Animals used:

Pregnant female rats were raised in a room under controlled light and temperature. The day of labour was considered as the first day of life. The offsprings were weighed on the first day of life for adjusting the dose of the drug. Then offsprings were kept with lactating mother up to sacrifice (day 10) or weaning (day 21). At birth the genital papilla of the male is rather larger than that of the female. A more reliable guide is the distance between the genital papilla and anus. It is greater in the male than in the female. Male rats were thereafter housed 4-5 per cage.

2- Drug used:

Estradiol Benzoate (E2B) which is present in the form of Folon ampouls obtained from Miser Pharmacy Company. On the first day of life 50 male rats were injected by single dose of the drug subcutaneously with the dose adjusted according to the groups. Similarly, 25 male rats were injected by normal saline alone.

Group A:

Twenty five male rats were injected subcutaneosly at the first day of life by 0.5 mg Estradiol Benzoate (equivalent to the therapeutic dose) /5 gm body weight (low dose).

Group B:

Twenty five male rats were injected subcutaneously at the first day of life by 1mg Estradiol Benzoate/ 5 gm body weight (high dose) ***(Kincl et al., 1963 and Brown-Grant et al., 1975).***

Group C (Control group):

Twenty-five male rats (control group) were injected subcutaneously at the first day of life by 0.1 cc normal saline/ 5 gm body weight.

Five rats from each group were killed on postnatal days 10, 22, 33, 45 and 60. The animals were anaesthetized with diethyl ether then the abdominal cavity was opened by lower midline abdominal incision to expose the testis. The location of the testis was identified in each rat and photographed at adult age by close up camera then they were removed and also photographed by close up camera and fixed in Bouin's solution for 24 hours to prevent its degeneration. After fixation; dehydration, clearing, embedding and staining of the testes were done.

1- Dehydration:

This was done by passing the fixed tissue through ascending grades of alcohol (70% to 100% ethanol concentration). However, alcohol does not act as a paraffin solvent so it is necessary to replace the alcohol with xylol.

2- Clearing:

The solvent routinely used for clearance was xylol. The alcohol-dehydrated tissue was passed through successive changes of xylol until all the alcohol has been replaced by xylol.

3- Embedding:

The cleared specimens were impregnated in 3 changes 2hs each for parassin wax (54-56°C) then embedded in hard parassin wax (56-58°C).

4- Sectioning:

Once the excess wax had been turned away, serial sections of "5" microns in thickness were obtained.

5- Staining and mounting:

Most of the stains are of aqueous media so the waxed sections require hydration. This was achieved by passing the sections through xylol to remove the paraffin, then through absolute alcohol to remove the xylol, and then through the water. The sections were ready for staining. The stain used was Haemotoxylin (for staining nucleus) and eosin (for staining cytoplasm).

After staining the sections were passed through a series of increasing strengths of alcohol and then xylol.

Staining:

Haematoxylin (Hx) and Eosin (E): ***(Lamberg and Rothstein, 1978)***

- Fixative
- Bouin's fluid
- Sections: Paraffin sections cut at 5 micormeters.

Solutions:

a- Erlich's Haematoxylin (Hx):

Haematolxylin (HX):	(20 gm)
Absolute alcohol	100 ml
Glycerin	100 ml
Distilled water	100 ml
Glacial acetic acid	10 ml
Potushalum	10 gm

The Hx- dissolves in alcohol before the other ingredients.

The stain was left to ripen for some weeks in a cotton plugged flask which was exposed to sunlight and air in a warm place. After ripening, it was stored in a tightly capped bottle.

b- Eosin (E):

Eosin Y (yellowish)	1.0 gm
Distilled water	100.0 ml

Eosin Y is readily soluble in water 1% aquous. Solution of Eosin was prepared.

Technique:

1- Sections were brought down to water.

2- The sections were stained in Erlich's Hx for 5 minutes.

3- Blued in tap water for 3 minutes.

4- Stained in 1% aqueous eosin solution for 1 minute.

5- Washed in water.

6- Dehyderated in alcohol.

7- Cleared in xylol and mounted in Canada balsam.

Results:

Nuclei: Stain	Blue
Cytoplasm: Stains	Pink

Finally, the sections were mounted in a drop or two of Canda balsm dissolved in xylol. Mounting eliminated refraction of light passing through the section *(Cormack, 1987).*

RESULTS

RESULTS

I- Morphological changes:

Control:

The testes were observed within the scrotum from the postnatal day 22 onward (Fig. 1) and their size increases with age (Figs. 3 & 4).

Treated:

In this study there was no descent of the testes into the scrotum and they remained intrabdominally (Fig. 2). The testicular size is less than that of the control (Figs. 3 & 4).

Fig. (1): A photograph of a 60 days old control male rat showing the testes are normally packed in the scrotum & they are of normal size (arrows).

Fig. (2): A photograph of a 60 days old male rat treated with high dose of estradiol benzoate (E2B) showing undescended testes which still present in the abdominal cavity (arrow). Notice also their small size in comparison to the control seen in Fig. (1).

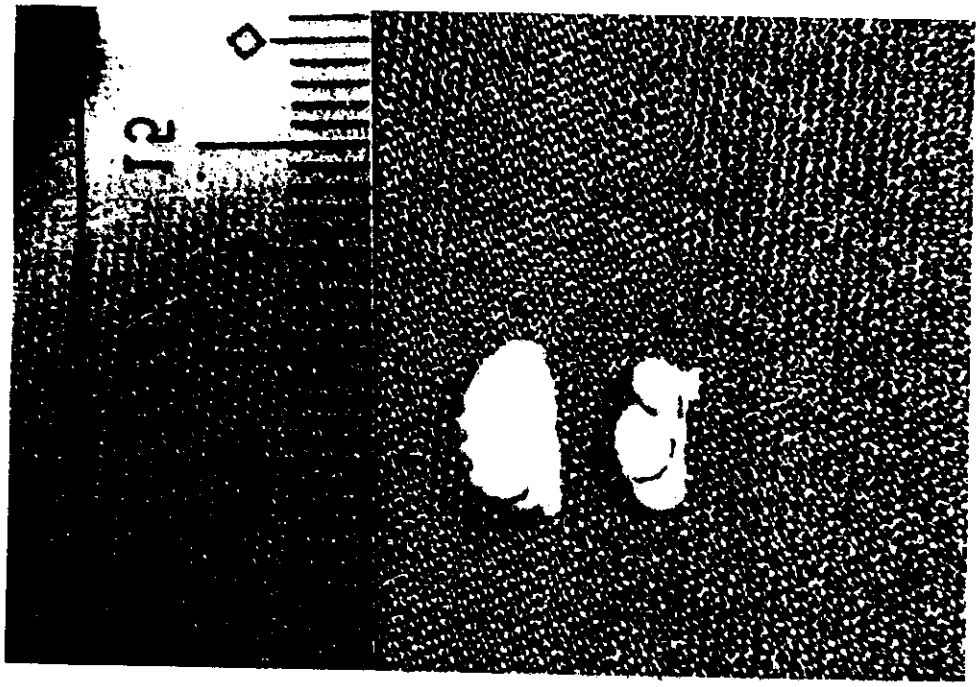

Fig. (3): **On the Lt:** A photograph of a 22 days old control male rat left testis showing normal size and shape (C).

On the Rt: A photograph of a 22 days old rat left testis treated with high dose estradiol benzoate (E2B) showing smaller size (t).

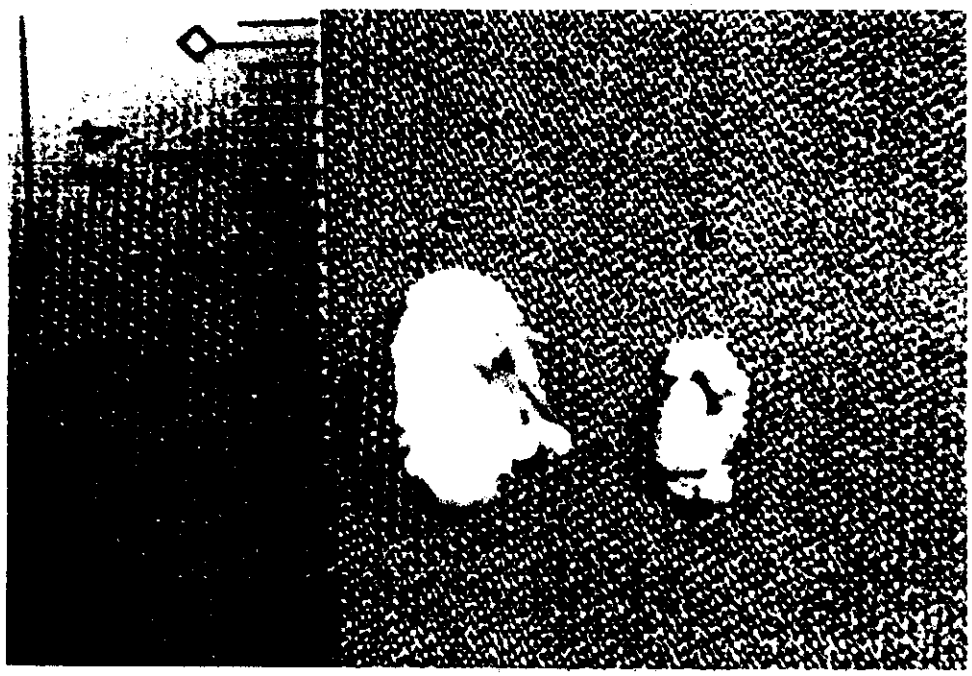

Fig. (4): **On the left:** A photograph of an adult control male rat (60 days old) left testis showing normal size (C).

On the right: A photograph of an adult 60 days old rat left testis treated with high dose of (E2B) showing smaller size (t).

II- Histological changes:

1- 10 days old rat:

Control:

The cross section of the testis shows seminiferous tubules with normal configuration (Fig. 5). The tubules are lined by two types of cells, the spermatogonia which are the larger cells and the tall columnar Sertoli cells in between. The Sertoli cells have poorly defined cytoplasm with ovoid pale vesicular nuclei (Fig. 6).

The rete testis shows a network of channels of varying size and shape (Figs. 7 & 8).

Treated:

Group A (low dose E2B):

In the cross section of the cranial part of the testis the seminiferous tubules show dilatation of their lumina (Fig. 9). The tubules are lined by normal Sertoli cells & spermatogonia (Figs. 10,11). In the cross section of the caudal part of the testis the tubules are less dilated (Figs. 9 & 12).

The rete testis show dilatation and connection between its channels (Fig. 13).

Group B (high dose E2B):

The cross section of the testis shows the seminiferous tubules with dilated lumina. Its the basement membrane is lined by Sertoli cells and spermatogonia like that of the control. The tubules are separated by interstitum (Figs.14 & 15).

The rete testis shows more dilatation and connection between its channels (Fig. 16).

Fig. (5): A photomicrograph of a 10-days control rat testis showing closely related seminiferous tubules separated by a little interstitium (it). The tubules show normal lumina (u) (Hx & E x 400).

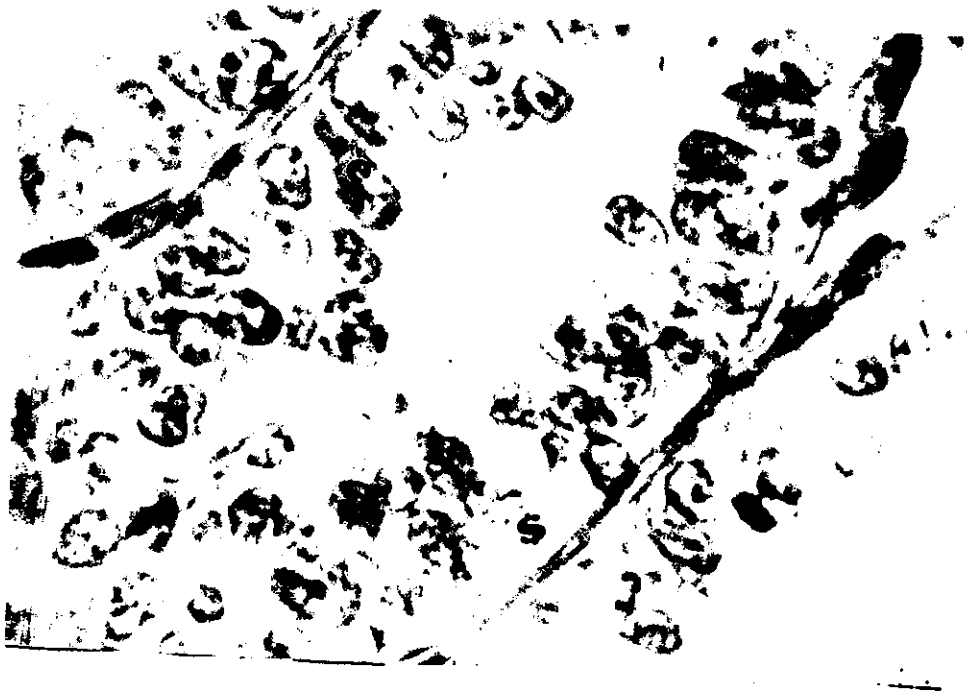

Fig. (6): A magnified photomicrograph of the previous section showing a closely related seminiferous tubules. The epithelium is formed of Sertoli cells (e): with poorly defined cytoplasme and ovoid vesicular nuclei and spermatogonia (s) (Hx & E X 1000).

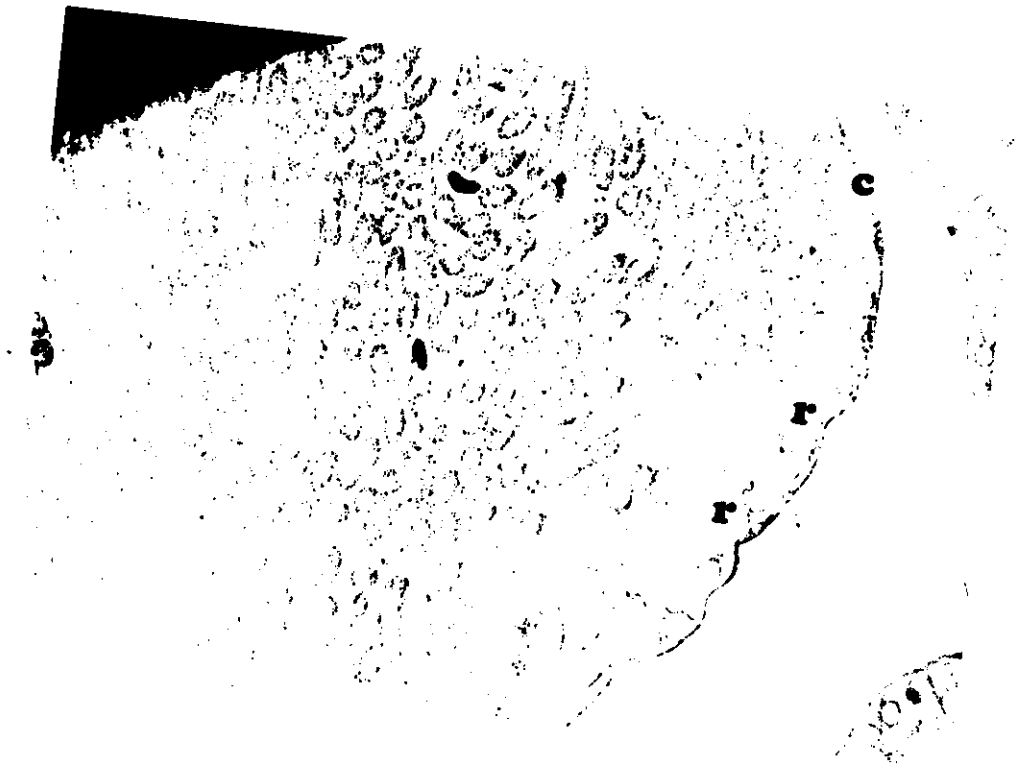

Fig. (7): A photomicrograph of a 10-days control rat showing rete testis (r) seen just deep to the capsule (c). they are of different size & shape, the cranial part of the testes lies adjacent to the rete testis whereas the a coudal part is away from it (Hx & E X 40)

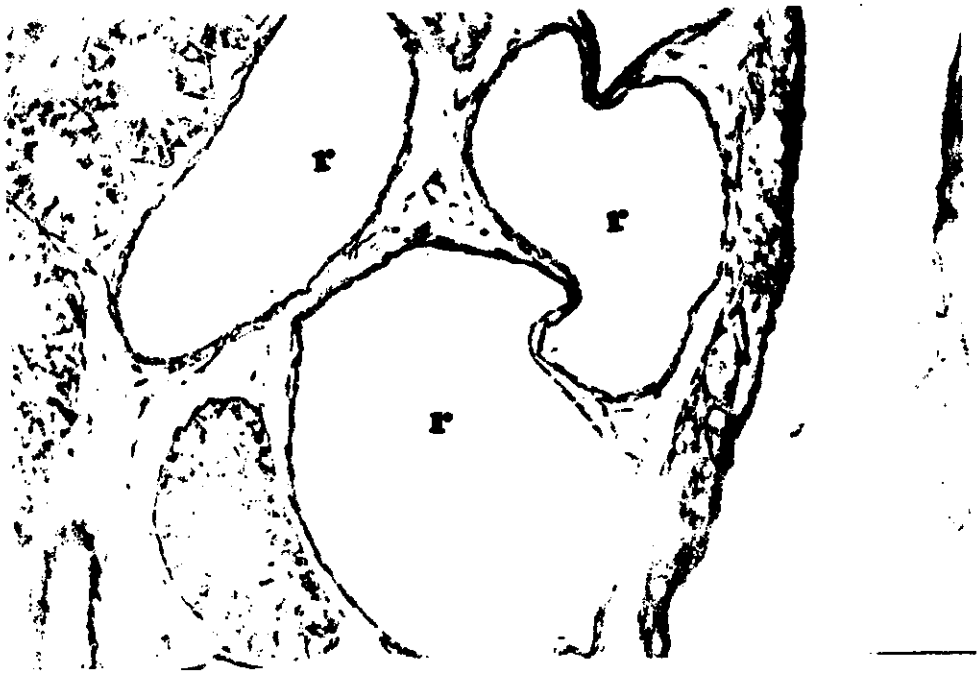

Fig. (8): A photomicrograph of 10-days control rat testis showing rete testis (r) which are of different shape and size.

(Hx & E X 200)

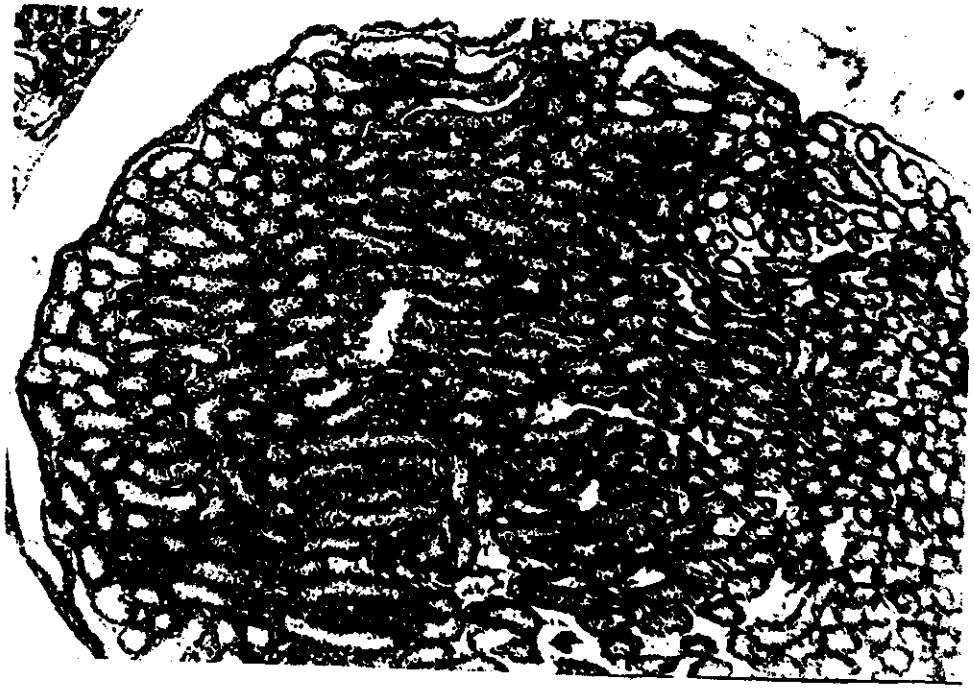

Fig. (9): A photomicrograph of a 10-days rat testis treated with a low dose of estradiol benzoate (E2B) showing testis and part of epididymis (ed). The cranial part of the testis (near epidymis) shows dilatation while the caudal part (*) is not dilated.

(Hx & Ex40)

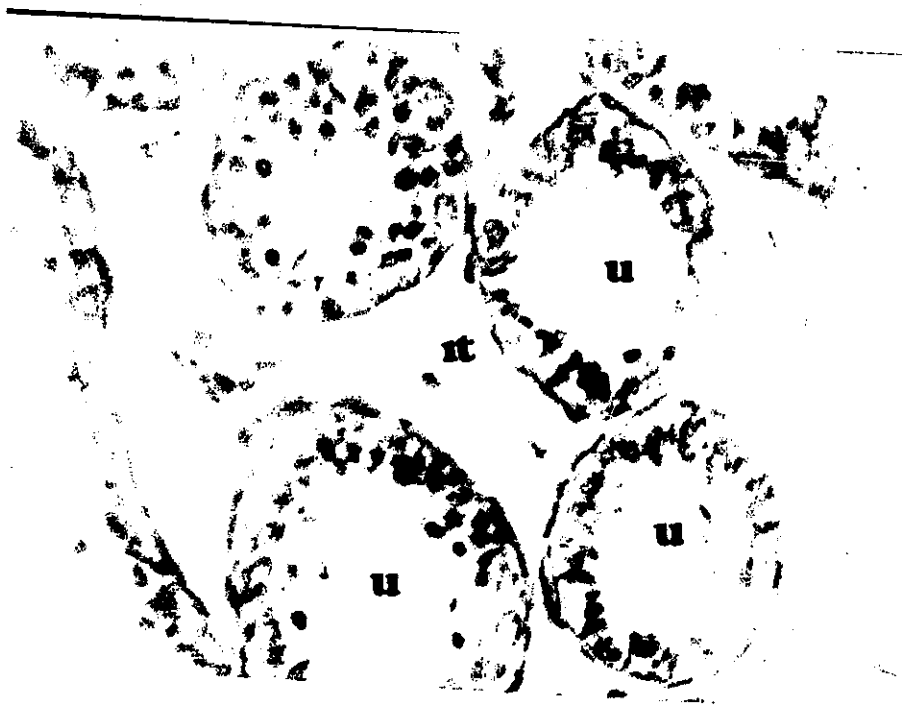

Fig. (10): A magnified photomicrograph of the cranial part of the previous section showing dilated seminiferous tubules (u). The tubules are separated by interstitium (it).

(Hx & E X 400)

Fig. (11): A magnified photomicrograph of the seminiferous tubules of the previous section showing Sertoli cells (e) and spermatogonia (s) (Hx & E X 1000).

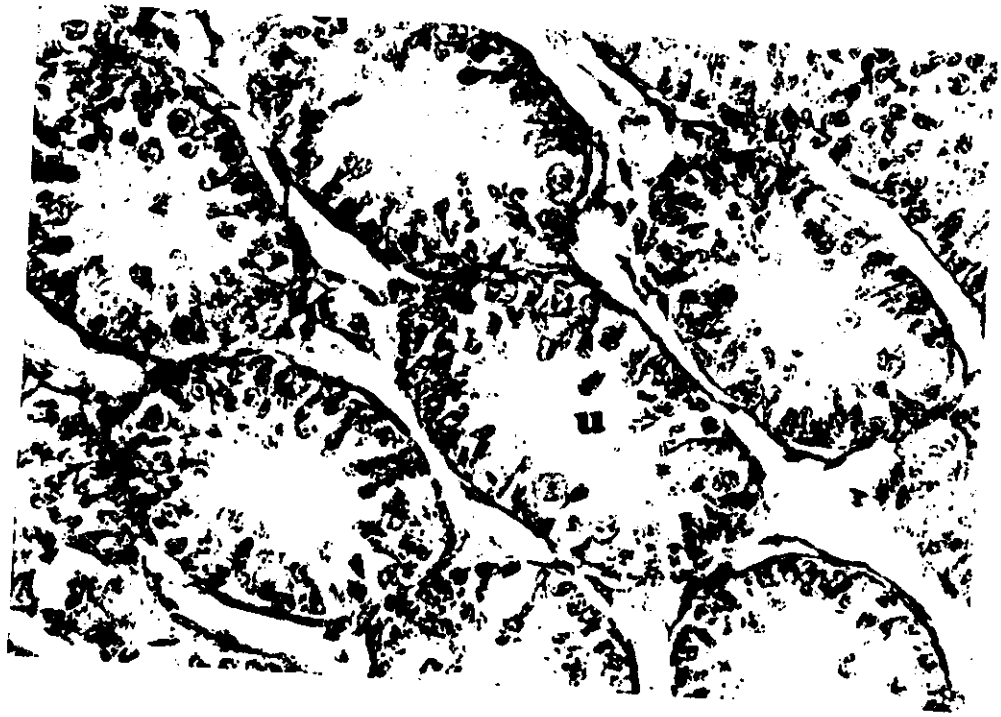

Fig. (12): A magnified photomicrograph of the caudal part of the Fig. (9) showing less dilated seminiferous tubules (u) and are separated by interstitium (it) (Hx & E X 400).

Fig. (13): A photomicrograph of a 10-days treated rat testis with a low dose of estradiol benzoate (E2B) showing dilated and connected channels (arrow) of rete testis (r).

(Hx & E X 200)

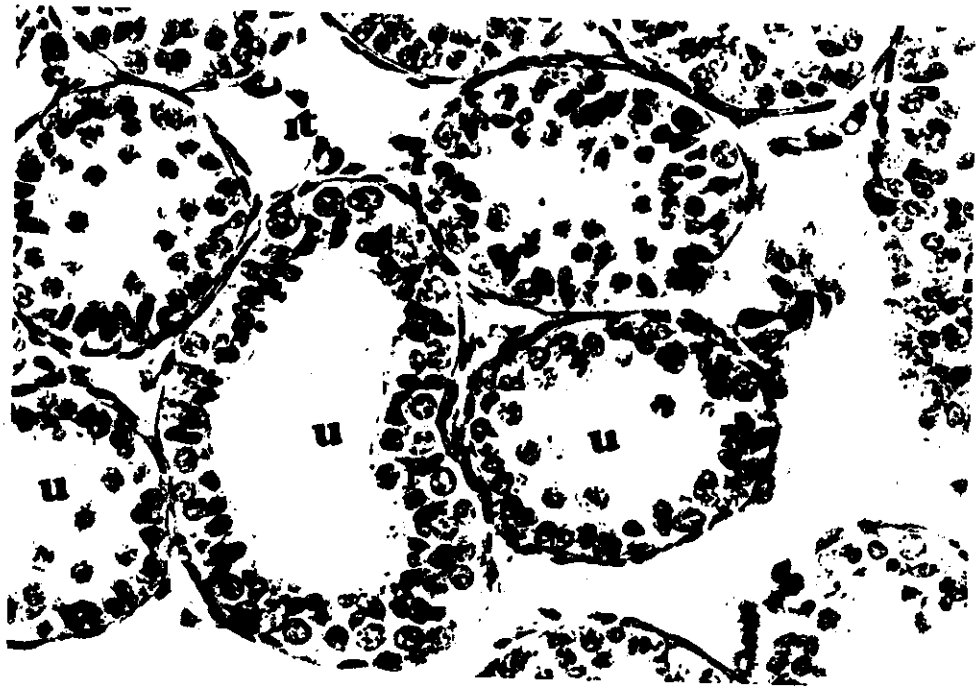

Fig. (14): A photomicrogaph of a 10-days rat testis treated with a high dose of (E2B) showing dilated seminiferous tubules (u) lined by sertoli (e) cells and spermatogenia (s) and are separated by interstitum (it).

(Hx & E X 400).

Fig. (15): A magnified photomicrograph of previous section showing seminiferous tubules (u). The tubules are lined by normal Sertoli cells (e) & spermatogonia (S) (Hx & E X 1000).

Fig. (16): A photomicrograph of a 10-days rat testis treated with a high dose of (E2B) showing more dilated connected channels (arrow) of rete testis (r)

(Hx & E X 200)

2- 22 days old rat:

Control:

The cross section of the testis shows closely packed seminiferous tubules. They are of normal shape and are well canalized. The tubules are separated by interstitum. Sertoli cells and spermatogonia are the same as those of previous age. The spermatogonia contain spherical nuclei. There are another type of cells which can be detected deeper to spermatogonia with a spherical nuclei containing chromatin crust (Fig. 17). These cells are the primary spermatocytes which form one or two layers (Fig. 18).

Treated:

Group A (low dose):

Cross section of the cranial part of the testis shows dilated seminiferous tubules. The tubules are lined by Sertoli cells and spermatogonia and its lumen contains sloughs of epithelium (Fig. 19, 20, 21). On the other hand, the cross section of the caudal part shows that the tubules are more or less like normal with no sloughes of epithelium into the lumen, the arrangement of the cells are more or less like normal and there are several raws of 1ry spermatocytes (Figs. 19, 22).

Group B (high dose E2B):

Cross section of the testis shows dilated seminiferous tubules which are separated by interstitum. The tubules are lined by normal Sertoli cells and spermatogonia. However, its lumen contains sloughs of the epithelium and inflammatory cells (Figs. 23, 24).

Fig. (17): A photomicrograph of a 22-days old control male rat testis showing adjacent seminiferous tubules which are well canalized (u) and separated by interstitium (it). The epithelium consists of Sertoli cells (e) and spermatogonia (s). At this age 1ry spermatocytes (p) can be detected. (Hx & E X 400)

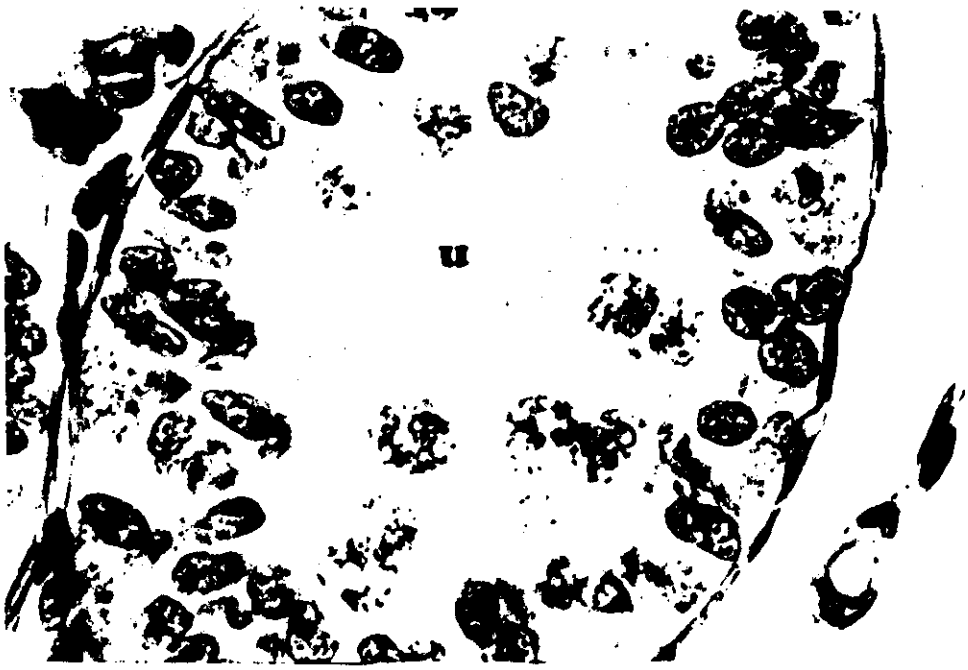

Fig. (18): A magnified photomicrograph of the previous section showing that the seminiferous tubules are well canalized (u) and their linning epithelium is formed of Sertoli cells (e) and spermatogonia (s).1ry spermatocyts (p) with mitotic figuers can be seen.

(Hx & E X 1000).

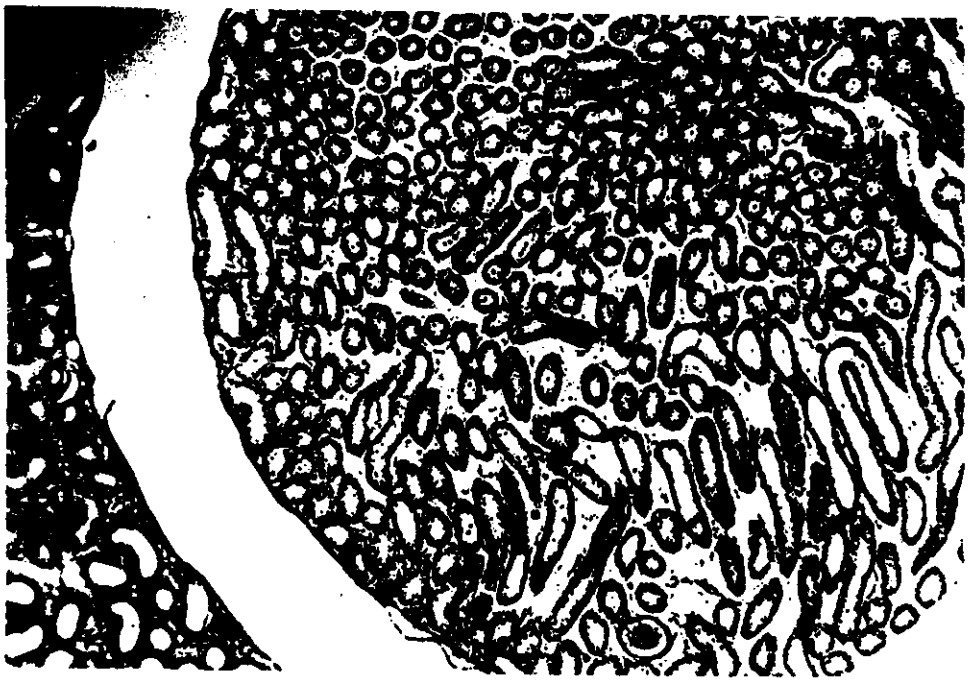

Fig. (19): A photomicrograph of a 22-days old rat testis treated with low dose of (E2B) showing testis & small part of epididymis (ed). The cranial part is near to epididymis while the caudal part away from it(*) (Hx & E X 40).

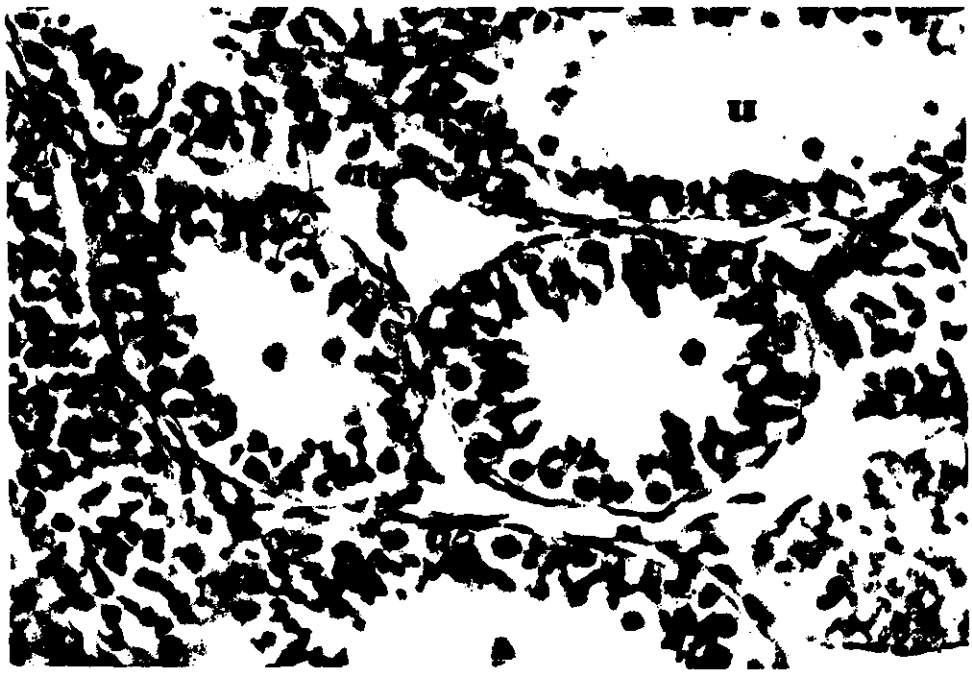

Fig. (20): A magnified photomicrograph of the cranial part of the previous section showing dilated seminiferous tubules (u). The tubules are separated by interstitium (it) (Hx & E X 400).

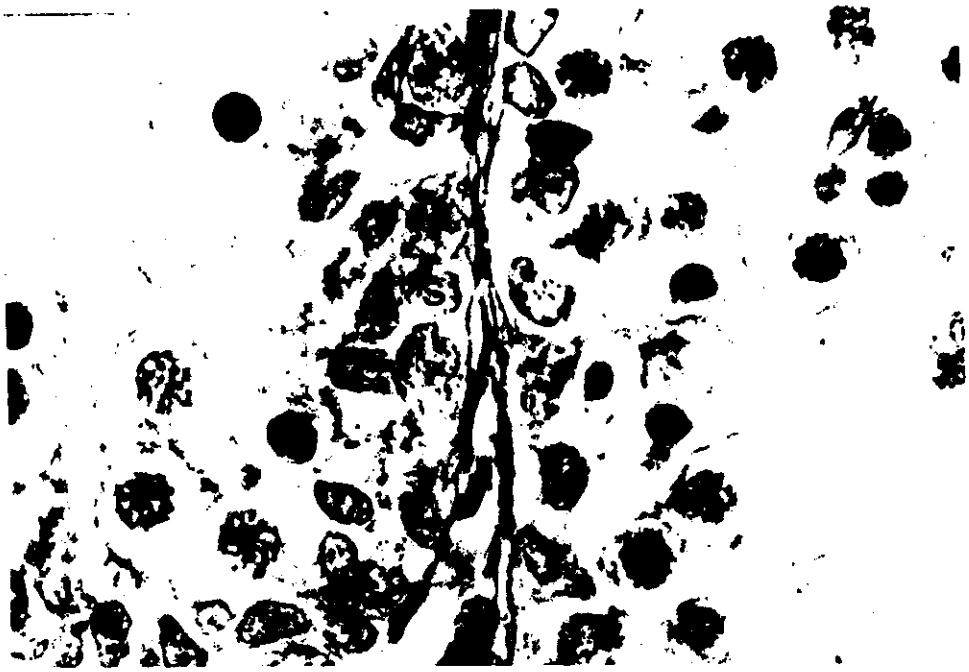

Fig. (21): A magnified photomicrograph of the previous section showing adjacent seminiferous tubules. Their lumen show sloughed epithelial cells (g) and their epithelium consists of Sertoli cells (e) & spermatogonia (S).
(Hx & E X 1000)

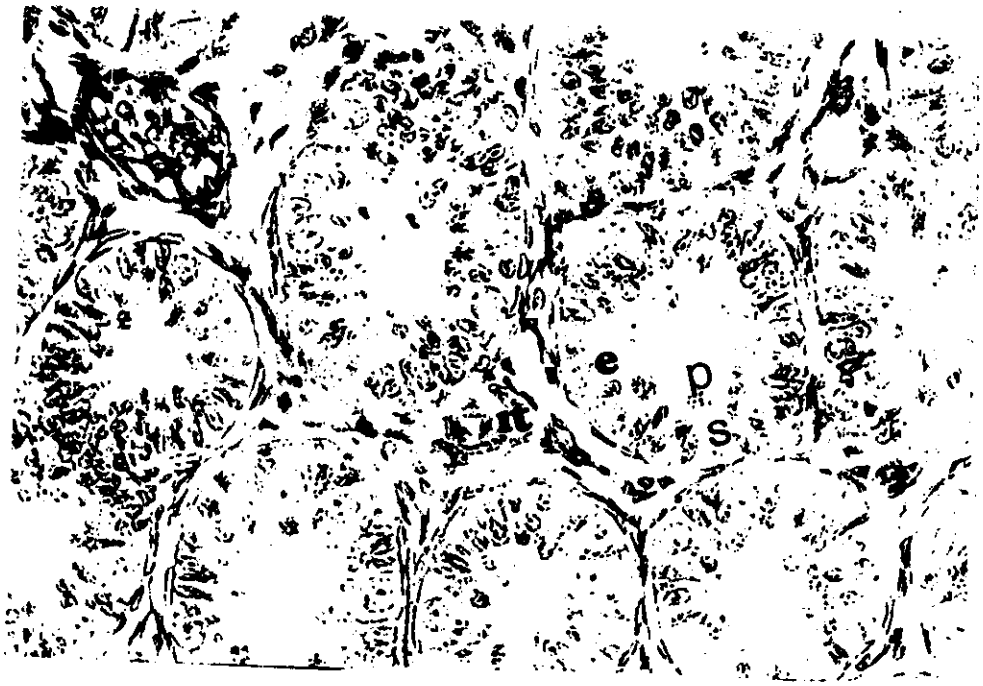

Fig. (22): A magnified photomicrograph the caudal part of (Fig. 19) showing seminiferous tubules with lining Sertoli cells (e) & spermatogonia (S). And there are no sloughes into lumen and there are 1ry spermatocytes (P), the tubules are separated by interstitum (it) (Hx & E X400).

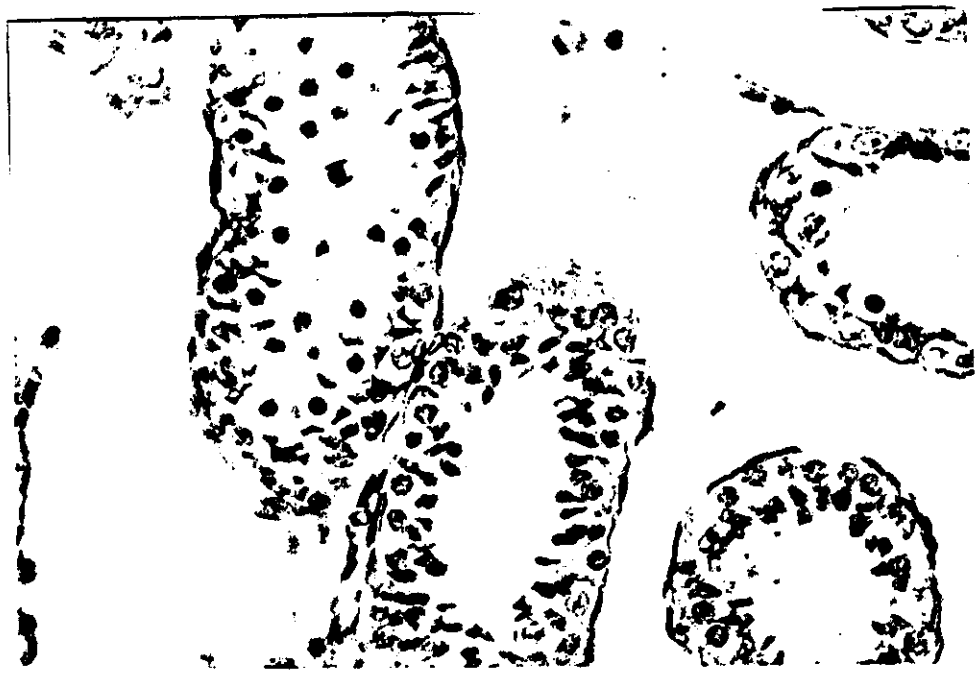

Fig. (23): A photomicrograph of a 22-days old rat testis treated with a high dose of (E2B) showing seminiferous tubules separated by dilated interstitum (it). The lumen of tubules is dilated and contains inflammatory cells (I) (Hx & E X 400).

Fig. (24): A magnified micrograph of the previous section showing seminiferous tubules which are lined by Sertoli cells (e) & spermatogonia (S). The lumen shows sloughed epithelium (g) and there are inflammatory cells (I). (Hx & E X 1000).

3- 33 days old rat:

Control:

Cross section of the testis shows seminiferous tubules with more canalization and gradual widening of their lumina. By this time, two types of spermatogonia can be detected. The first is type A cells with deeply stained spherical nuclei containing centrally located chromatin mass. The second is type-B cells with pale spherical or ovoid nuclei. The primary spermatocytes form a several layers which occupy the center of the tubules and their nuclei are deeply stained. Sperms and spermatids could not be detected at this age (Figs. 25, 26).

Treated:

Group A (low dose E2B):

Cross section of the cranial part of the testis shows seminiferous tubules separated by interstitum. The tubules are lined by spermatogonia and Sertoli cells only. Its lumen contains sloughs and inflammatory cells. The 1ry spermatocytes could not be detected inside the tubules (Figs. 27, 28, 29). Cross section of the caudal part shows that the tubules are more or less like that of the control. There are normal lining epithelium and there are no sloughes into the lumen or inflammatory cells. Also, there are evident 1ry spermatocytes which are not seen in the cranial part (Fig.30).

Group B (high dose E2B):

Cross section of the testis shows dilated seminiferous tubules with many inflammatory cells in the lumen. There is impairment of spermatogenesis which is evidenced by presence of sloughs of cells into the lumen. The linning epithelium of the tubules consists of Sertoli cells and spermatogonia (Fig. 31, 32).

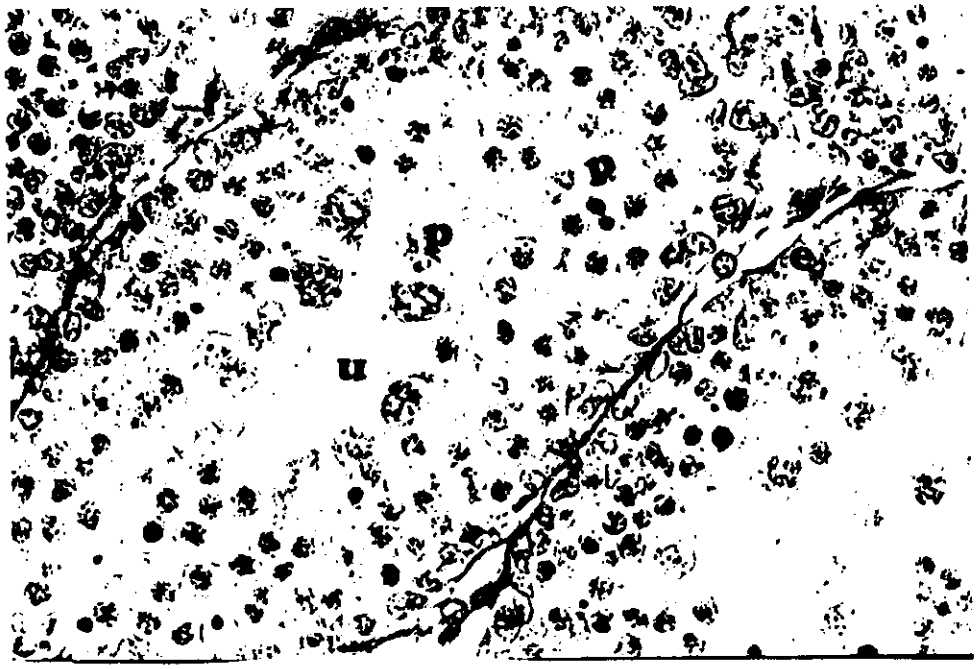

Fig. (25): A photomicrograph of a 33-days old control rat testis, showing well canalized tubules (u). The tubules are separated by interstitium. They contain spermatogonia (S), Sertoli cells (e) on the basement membrane and there are 1ry spermatocytes (P) which are present in several raws.

(Hx & E X 400)

Fig. (26): A magnified photomicrograph of the previous section showing seminiferous tubules, lined by Sertoli cells (e) & spermatogonia type A (Sa), spermatogonia type B (Sb) and primary spermatocytes (P). No spermatids or sperms could be detected (Hx & E X 1000).

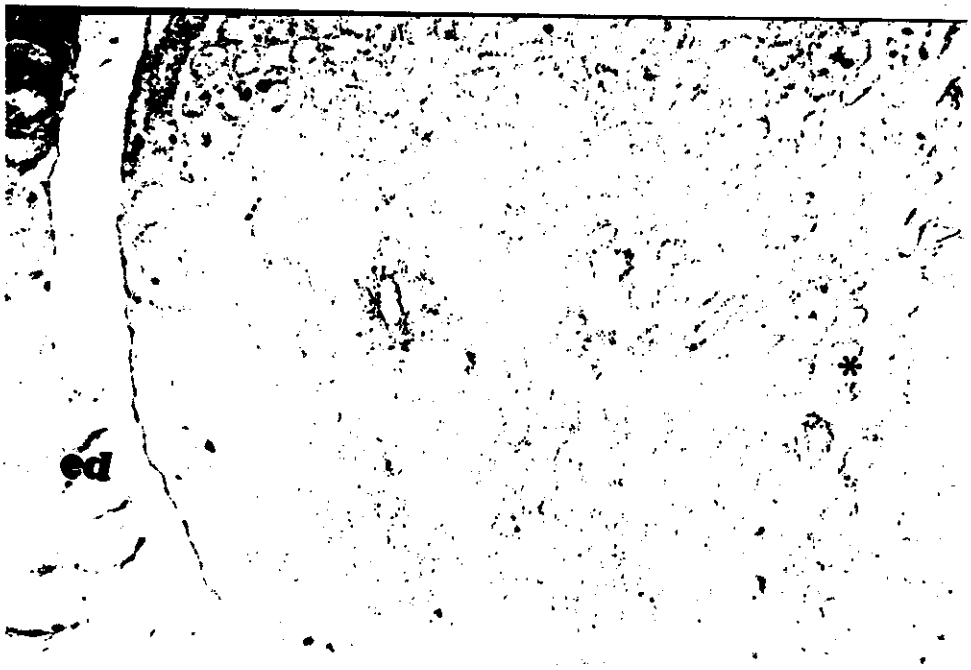

Fig. (27): A photomicrograph of a 33-days old rat testis treated with a low dose of (E2B) showing testis & small part of epididymis (ed). The cranial part near epididymis while caudal part away from it (*) (Hx & E X 40).

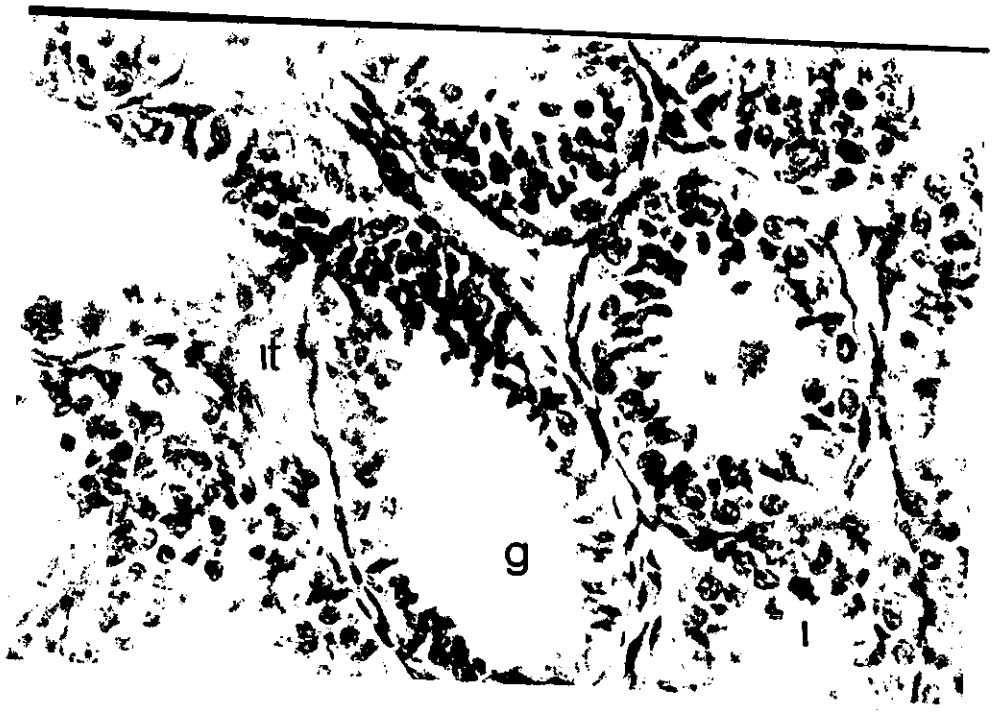

Fig. (28): A magnified photomicrograph of the cranial part of the previous section near the epididymis showing dilated seminiferous tubules with sloughes in the lumen (g) and inflammatory cells (I) separated by interstitium (it) (Hx & E X 400).

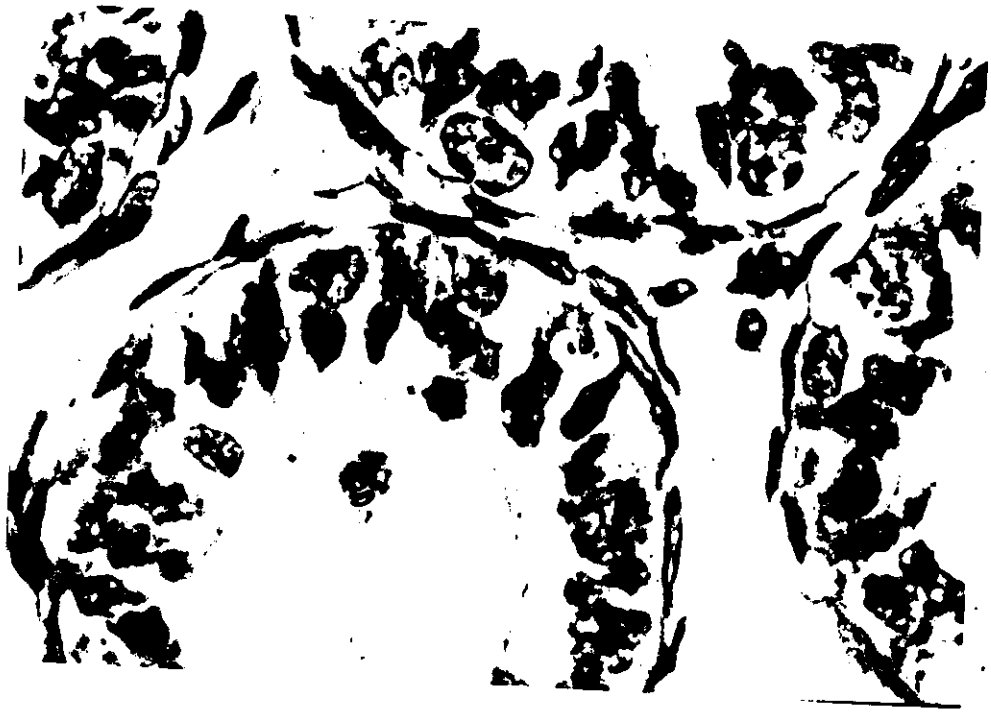

Fig. (29): A magnified photomicrograph of the previous section showing dilated seminiferous tubules which show sloughs (g) inside the lumen & lined by Sertoli cells (e) & spermatogonia (S). The tubules are separated by interstitum (it) (Hx & E X 1000).

Fig. (30): A magnified photomicrograph of the caudal part of (Fig. 27) showing seminiferous tubules which are less affected than that of cranial part as evidenced by presence of Sertoli cells (e), spermatogonia (S) there are 1ry spermatocytes (P) (Hx & E X 400).

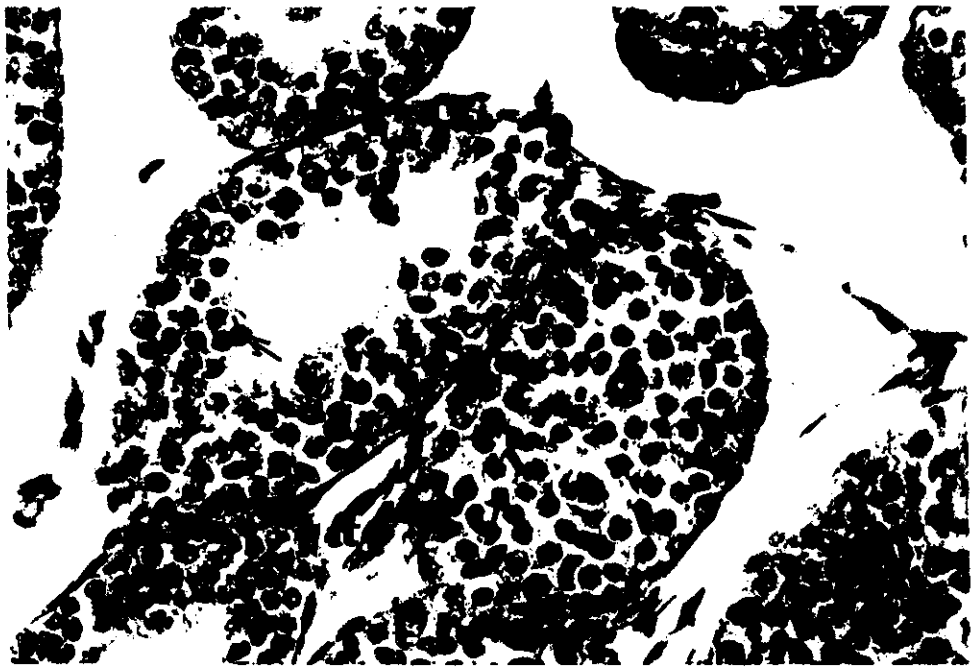

Fig. (31): A photomicrograph of a 33-days old rat treated with high dose (E2B) showing dilated seminiferous tubules with inflammatory cells (I) and separated by interstitium (it). The dilated lumen of one of the tubules (arrow) is filled with inflammatory cells (Hx & E X 400).

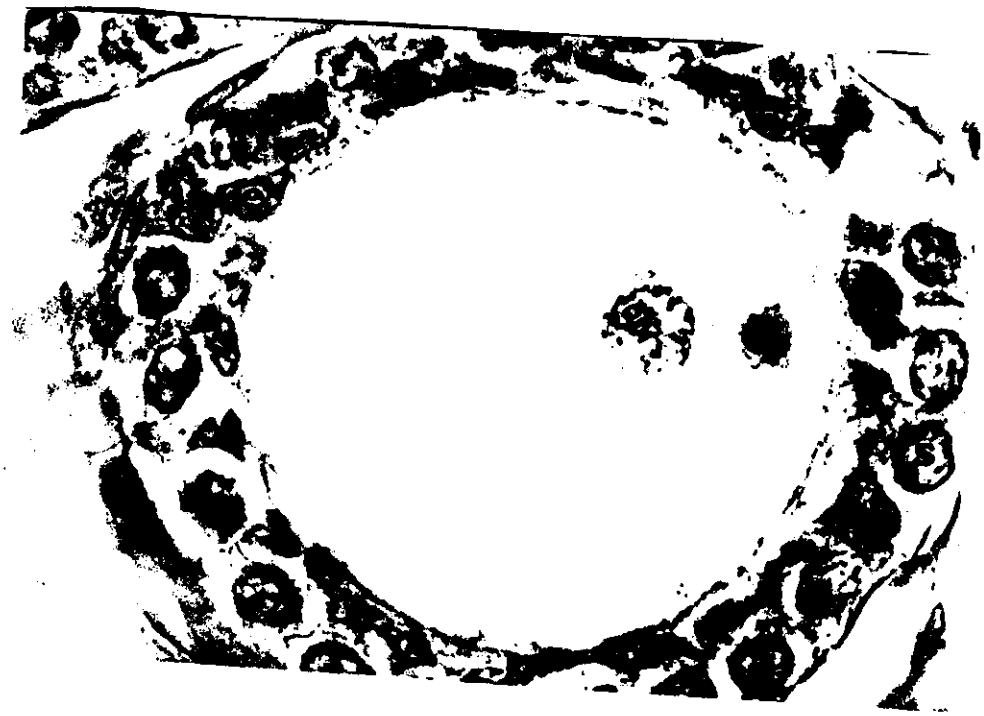

Fig. (32): A magnified photomicrograph of the previous section showing much dilated seminiferous tubules. The epithelium showing only Sertoli cells (e) & spermatogonia (S) with sloughes inside the dilated lumen (Hx & E X 1000).

4- 45 days old rat:

Control:

Cross section of the testis shows seminiferous tubules with normal arrangement of cells. The epithelium of seminiferous tubules is formed of Sertoli cells and spermatogonia. Sertoli cells which extend deeply between the raws of the spermatogonia extending from basement membrane toward the lumen of the tubule. All the series of the spermatogenic epithelium are clear. Spermatogonia resting on the basement membrane formed of 1-2 rows followed by primary spermatocytes which occupy the middle zone of the tubules followed by spermatids near the lumen, still, sperms could not be seen at this age (Figs.33 & 34).

Treated:

Group A (low dose E2B):

Cross section of the cranial part of the testis shows the seminiferous tubules separated by interstitum and they are lined by spermatogonia and Sertolic cells. There are impairment of spermatogenesis with the presence of sloughes and inflammatory cells (Figs. 35, 36 & 37). The cross section of the caudal part of the testis shows less affection and more or less like normal tubules as evidenced by the presence of normal arrangement of the cells. Sertoli cells, spermatogonia and primary spermatocytes are seen in several raws (Fig. 38).

Group B (high dose E2B):

Cross section of the testis shows that the seminiferous tubules presented more affected tubules. There are inflammatory cells and sloughed epithelial cells. The tubules are lined by Sertoli cells spermatogonia and separated by interstitum. There are no primary spermatocytes or spermatids (Fig. 39 and Fig. 40).

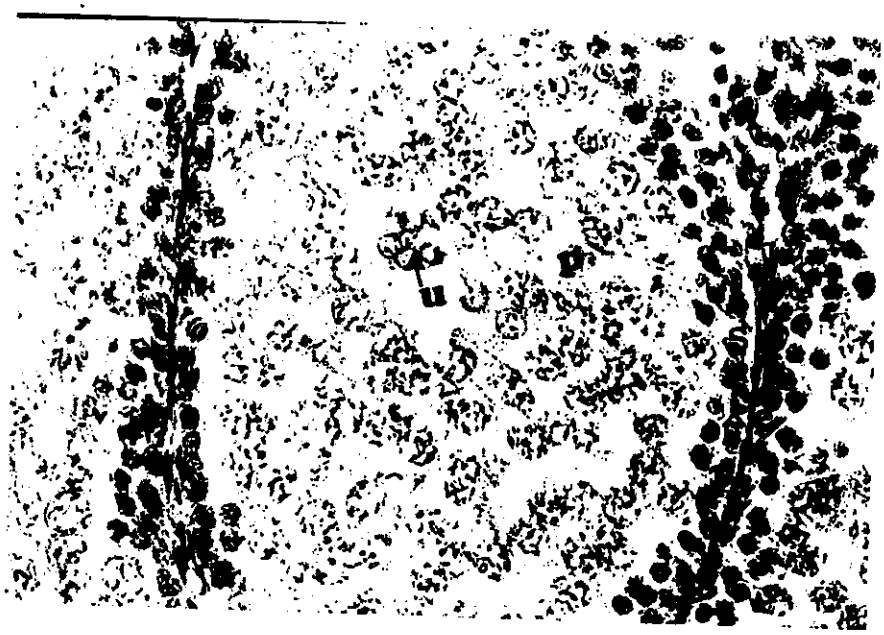

Fig. (33): A photomicrograph of a 45-days old control rat testis showing adjacent seminiferous tubules. The well apparent tubule shows spermatogonia (S), Sertoli cells (e) and primary spermatocytes (P) in several rows and spermatids (arrows) near the lumen (U). No sperms could be detected (Hx & E X 400).

Fig. (34): A magnified photomicrograph of a previous section showing a seminiferous tubules containing 1ry (P) spermatocytes in several raws and spermatids (d). No sperms could be detected (Hx & E X 1000).

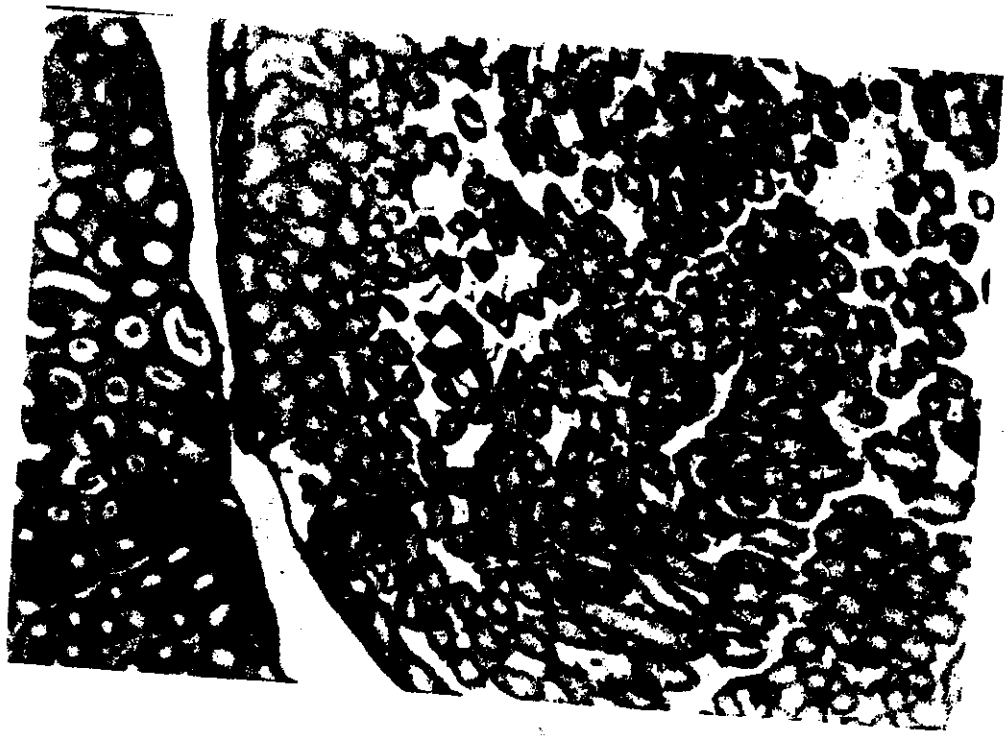

Fig. (35): A photomicrograph of a 45-days old rat testis treated with a low dose of (E2B) showing a small part of epididymis (ed). The cranial part is near to epididymis while the caudal part away from it (*) (Hx & E X 40).

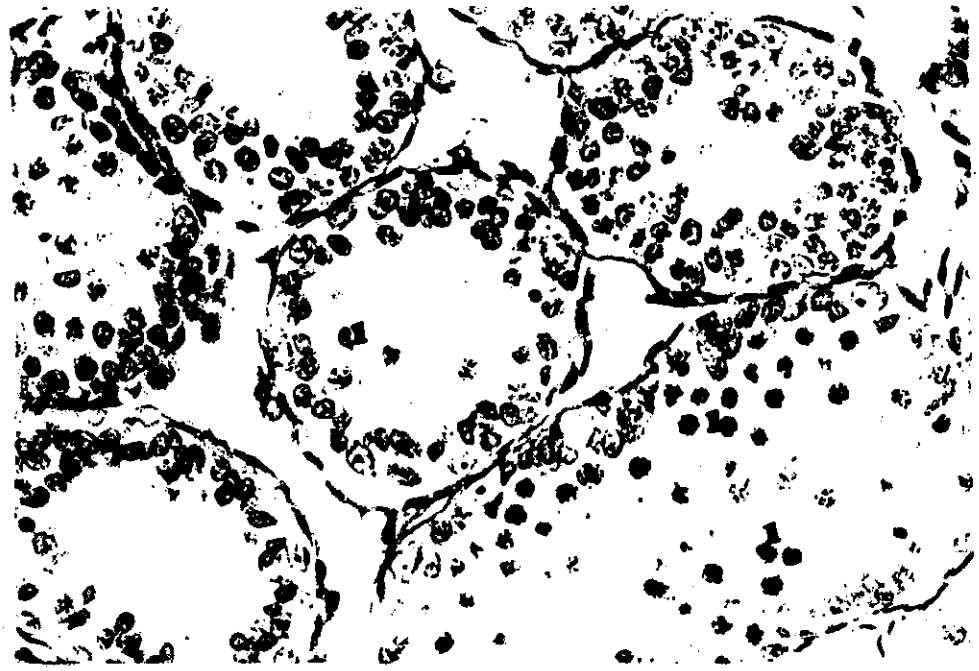

Fig. (36): A magnified photomicrograph of the cranial part of the previous section showing seminiferous tubules with inflammatory cells (I) into the lumen (Hx & E X 400).

Fig. (37): A magnified photomicrograph of the previous section showing seminiferous tubules with inflammatory cells (I) and sloughes into lumen. The linning epithelium is formed of Sertoli cells (e) & spermatogonia (S). (Hx & E X 1000).

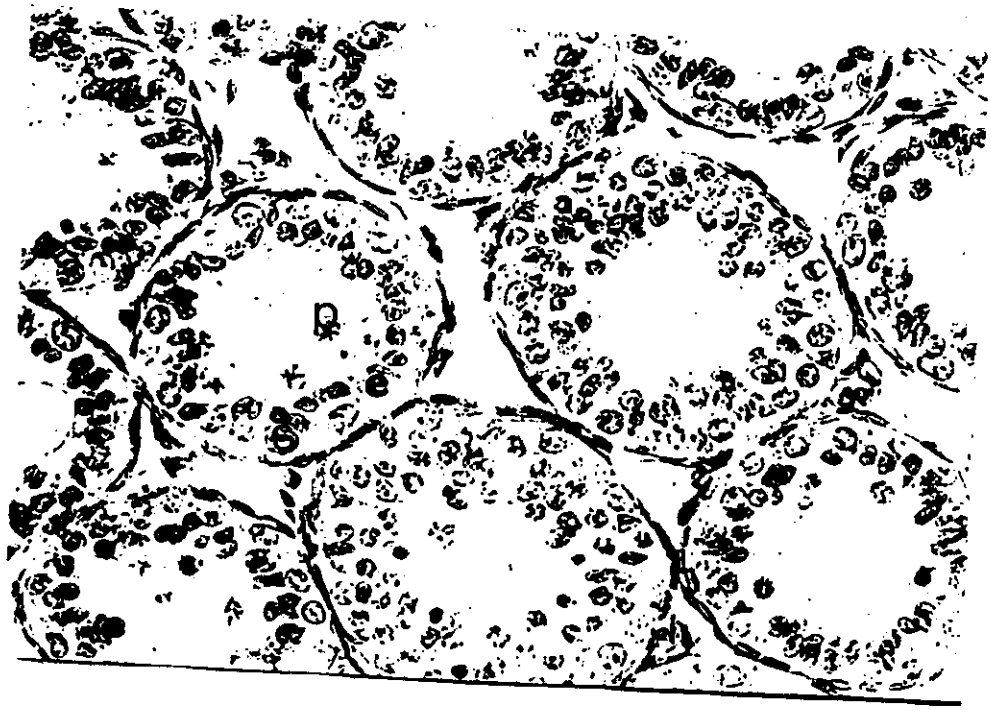

Fig. (38): A magnified photomicrograph of the caudal part of Fig. (35) showing less affected seminiferous tubules as evidenced by the presence of apparently normal tubules with linning Sertoli cells (e) & spermatognia (S) and there are primary spermatocytes (P). No spermatids or sperms could be detected (Hx & E X 400).

Fig. (39): A photomicrograph of a 45-days old rat treated with a high dose of (E2B) showing seminiferous tubules separated by interstitum (it) and show inflammatory cells (I) and sloughes of epithelial cells (g).

(Hx & E X 400)

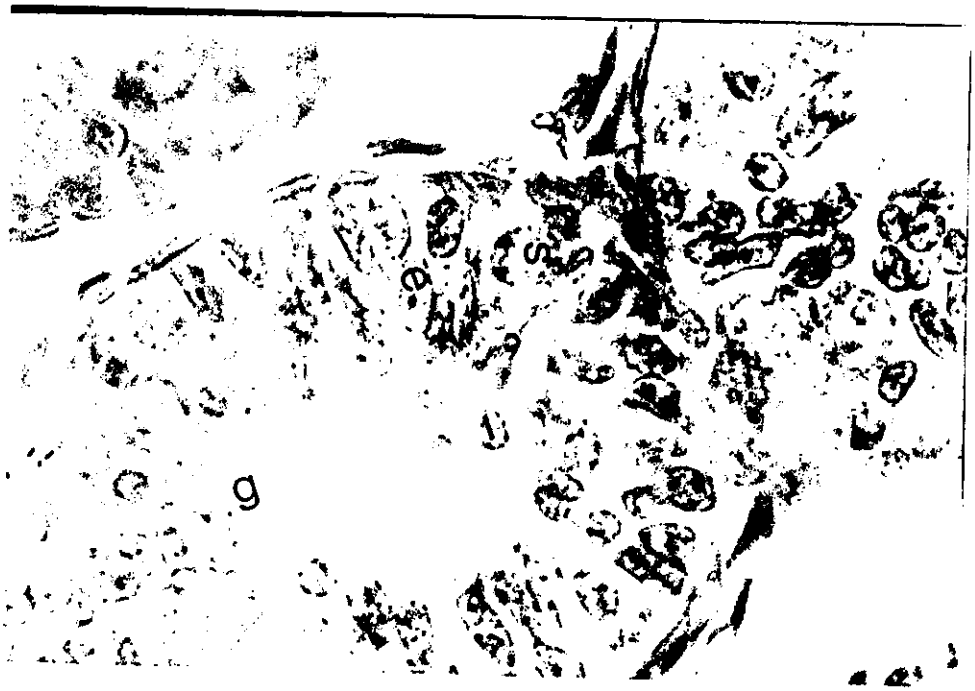

Fig. (40): A magnified photomicrograph of the previous section showing seminiferous tubules. There are sloughed epithelium (g) & inflammatory cells (I) into their lumen. The tubules are lined by Sertoli cells (e) & spermatogonia (S). There are no 1ry spermatocytes, spermatids can be seen.

(Hx & E X 1000)

5- 60 days old rat:

Control:

Cross section of the testis shows seminiferous tubules which are more or less oval in shape and closely packed together (Fig. 41). The tubules are lined by spermatogonia and Sertoli cells. The spermatogonia are arranged in rows. The spermatogonia are found in the basal layer of the tubules, with a dark rounded nuclei. The primary spermatocytes occupy the middle zone of the germinal epithelium. The secondary spermatocytes are short lived and therefore rarely seen. The spermatids are smaller than the primary spermatocytes and present in groups near the tubular lumen. The Sertoli cells rest on the basement membrane of the tubules in between the germinal cells. The sperms fill the lumen and are attached by their heads to the apices of Sertoli cells (Figs. 42 & 43).

Group A (low dose E2B):

Cross section of the cranial part of the testis shows seminiferous tubules with impairment of the spermatogenesis. There are inflammatory cells and sloughes inside the lumen. The tubules are lined by sertoli cells and spermatogonia (Figs. 44, 45 & 46). The cross section of the caudal part shows less affected tubules but spermatogenesis still impaired as there are no sperms seen. There are only, Sertoli cells, spermatogonia and 1ry spermatocytes (Fig. 47).

Group B (high dose E2B):

Cross section of the testis shows seminiferous tubules, separated by interstitum with dilated lumen. The tubules show spermatogenic impairment. The tubules are lined by Sertoli cells and spermatogonia only. Here the number of Sertoli cells are much more than spermatogonia. There are no sperms untile this age (Figs. 48 & 49).

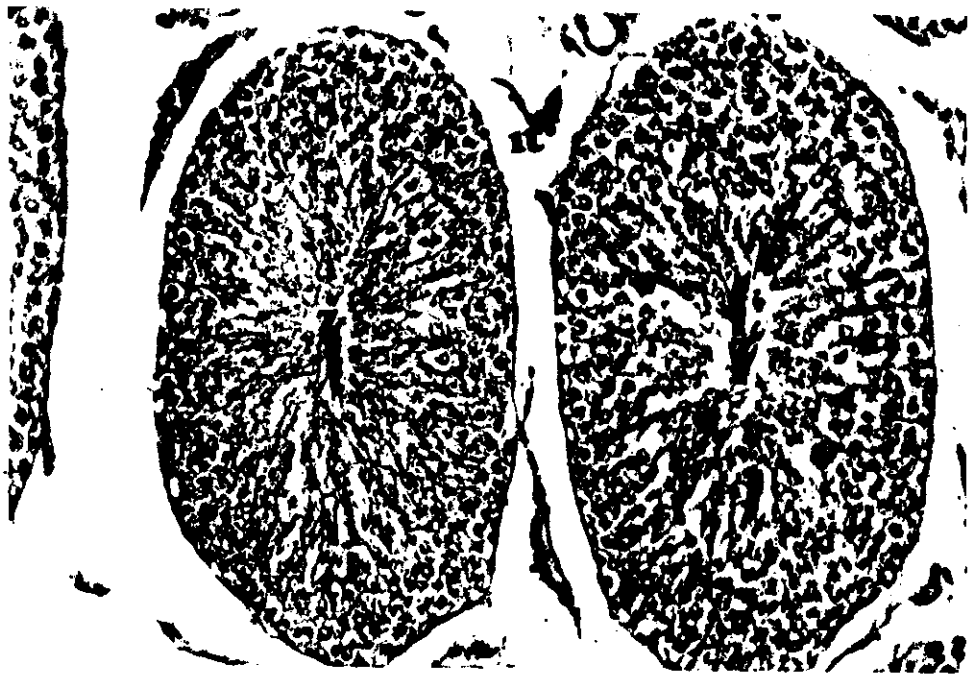

Fig. (41): A photomicrograph of a 60-days old control rat showing two seminiferous tubules which are separated by interstitium (it).Sperms are seen inside the lumen of the tubules (z) (Hx & E x200).

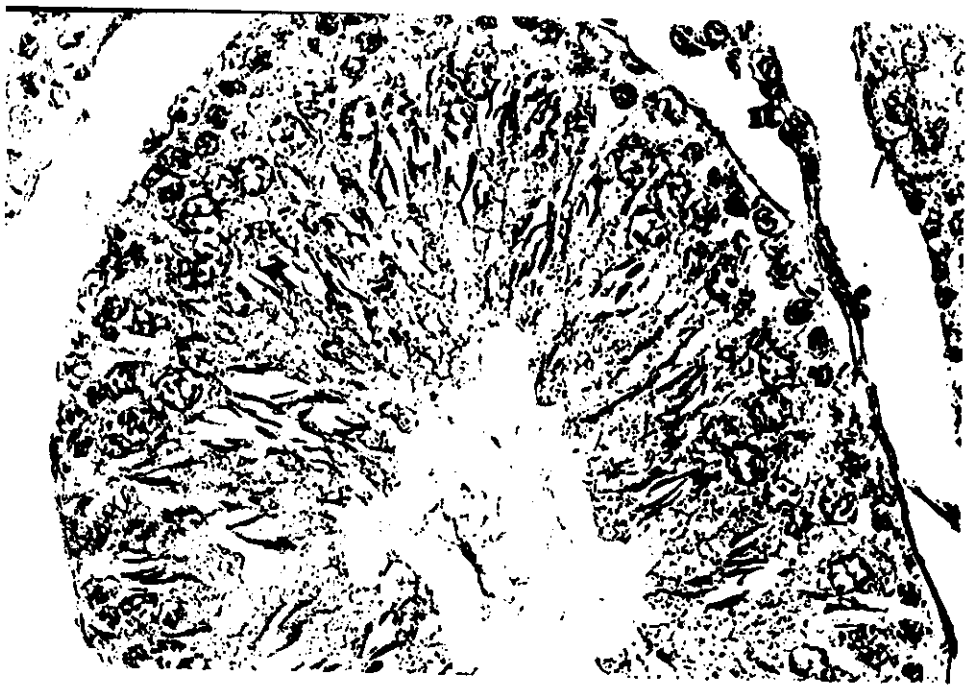

Fig. (42): A photomicrograph of a 60-days old control rat showing one seminiferous tubule and part of other which are separated by interstitium (it). Its linning epithelium is formed of Sertoli cells (e), spermatogonia (S) and there are 1ry spermatocytes (P) and sperms (Z) (Hx & E x400).

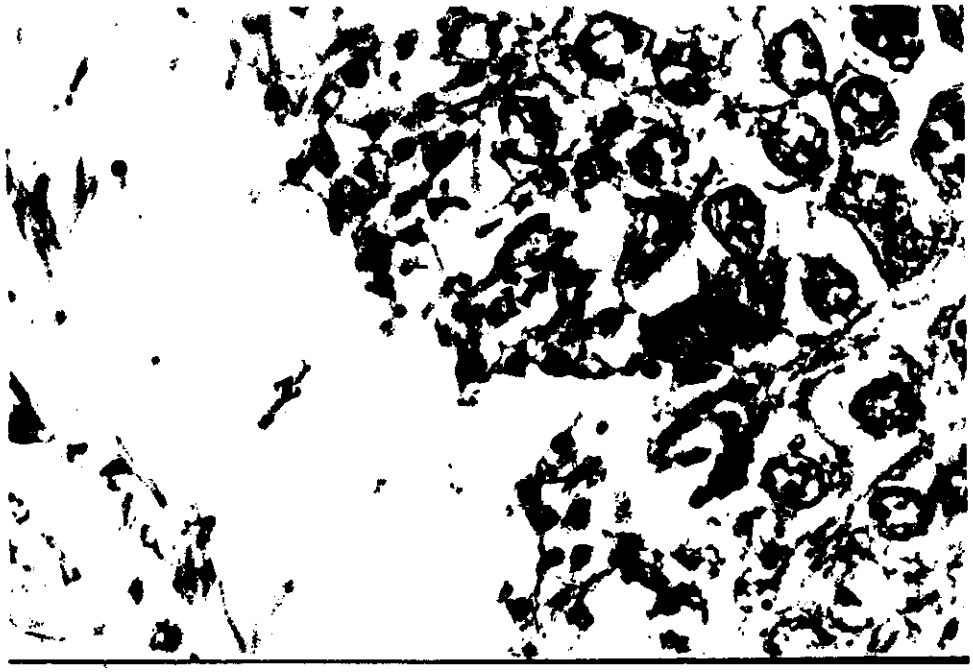

Fig. (43): A magnified photomicrograph of the previous section showing seminiferous tubule with 1ry spermatocyte (P), spermatids (d) Sperms (z) in the lumen (Hx & E X 1000).

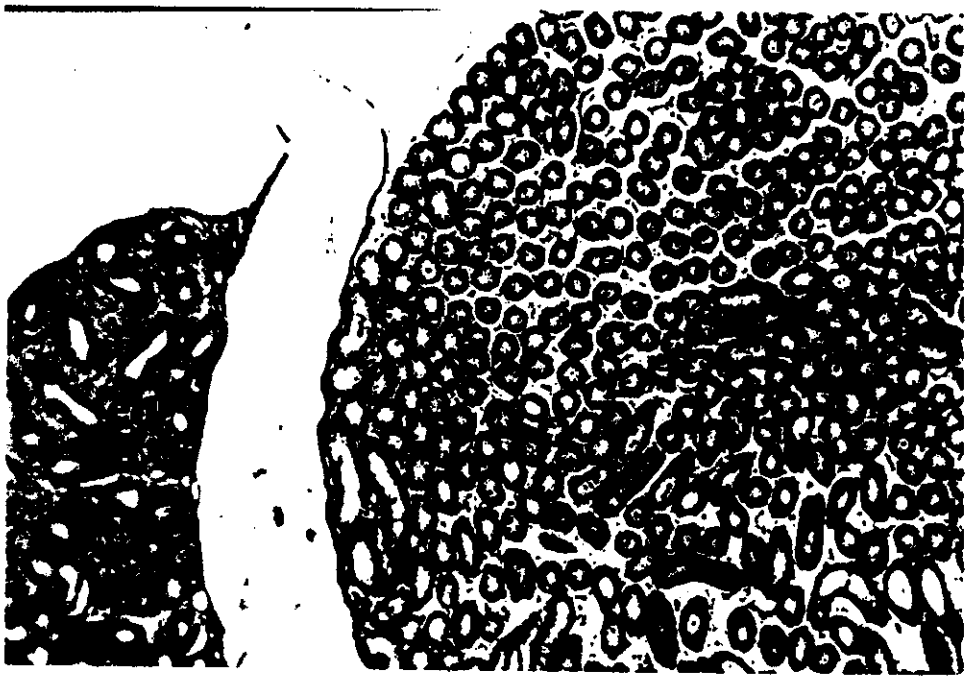

Fig. (44): A photomicrograph of a 60-days old rat treated with a low dose of (E2B) showing testis & small part of epididymis (ed). The cranial part is near to epididymis while the caudal part is away from it (*) (Hx & E X 40).

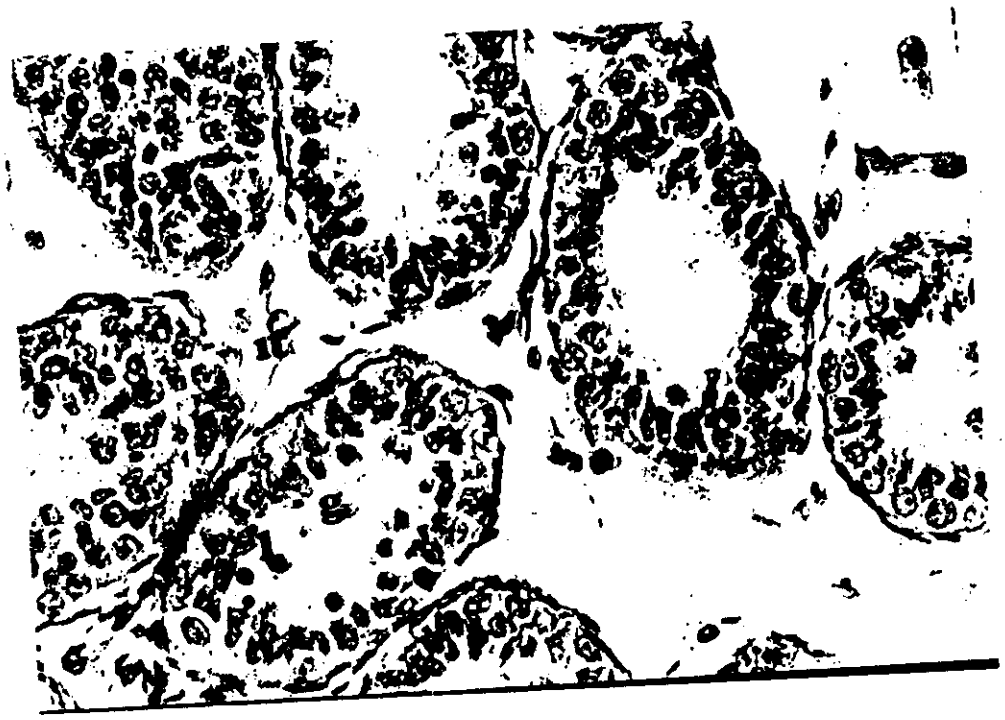

Fig. (45): A magnified photomicrograph of the cranial part of the previous section showing seminiferous tubules separated by interstitum (it) and there are sloughes of epithelium (g) and inflammatory cells (I) (Hx & E X 400).

Fig. (46): A magnified photomicrograph of the previous section showing seminiferous tubules with inflammatory cells (I). The epithelium is formed of Sertoli cells (e) & spermatogonia (S) only, 1ry spermatocyte, Spermatids and sperms could not be detected (Hx & EX1000).

Fig. (48): A photomicrograph of a 60-days old rat treated with a high dose of (E2B) showing dilated (u) seminiferous tubules separated by interstitum. There Sertoli cells (e) and spermatogonia (s) on the basement membrane but 1ry spermatocytes spermatids or sperms could not be detected (Hx & E X 400).

Fig. (49): A magnified photomicrograph of the previous section showing dilated seminiferous tubules with sloughs of epithelium (g) into the lumen, impaired spermatogenesis as evidence by absence of sperms until this age. Only the linning epithelium is formed of Sertoli cells (c) & spermatogonia (S) (Hx & E X 1000).

DISCUSSION

DISCUSSION

Disorders of development of the reproductive tract in the male fetus and maldescent of the testis are increasing in incidence in the last years *(Giwercman & Skakkebaek, 1992; Sharp & Skakkebaek, 1993).*

Sharp & Skakkebaek (1993) suggest that there has been generally an increased human exposure to estrogen over the past 50 years. This has been caused by change in the diet and body composition (more fat) which led to more exposure of women to their own estrogen *(Adlercreutz, 1990)* and increased exposure to environmental estrogens as pollutant chemicals *(Hileman, 1993).*

It remains unknown whether the level of human exposure to such estrogenic chemicals is sufficient to exert adverse effect on the development of the reproductive system of the early male fetus or the developing child. However, the increasing prevalence of such disorders in man provides support for this possibility *(Sharp & Skakkebaek, 1993).*

Sharp (1994) decided that, irrespective of whether increased human exposure to estrogens is responsible for the increasing incidence of male reproductive abnormality in adult human is predetermined by events in fetal life and childhood.

In the present study, the cross section of the treated rat testis showed dilatation of the rete testis which started early in life and also showed spermatogenics disruption. It showed dilatation of the lumen of the seminiferous tubules together with the presence of epithelial sloughs and inflammatory cells. Morphological studies showed small-sized undescended testes which were arrested intra-abdominally. These findings were also reported by ***Vitale et al. (1973) and Chemes et al. (1976) and Russel et al. (1989);*** they added that, the rats treated with low dose estrogen showed descent of testis at 60 days of age, but still smaller in size in comparison to the control of the same age.

Exposure of male rodents to estrogens in the early postnatal life is useful for understanding the mechanisms involved in the effects on the human reproductive tract, whose mothers either received diethyl stilbestrol (DES) which is a potent estrogen or were exposed to environmental estrogens during pregnancy ***(Gill et al., 1979; Newbold et al., 1985; Sharp & Skakkebaek, 1993).***

This effect on the reproductive tract is secondary to that on growth and structure of the seminiferous tubules and rete testis which have been observed in both early postnatal estrogenized rats ***(Orgebin-Crist et al., 1983; Newbold et al., 1986 & Greco et al., 1993)*** and male feti exposed in utero to DES ***(Griffin and Wilson, 1994).***

The mechanisms by which estrogen exert these effects on the testis are:

A- Estrogen-induced alteration on the hypothalamic pituitary-gonadal axis leads to different effects on spermatogenesis:

1- Long-lasting suppression of this axis may cause a persistant inhibition of the spermatogenesis *(Arai et al., 1983; Bellido et al., 1990 and Pinilla et al., 1992).*

2- A decrease in testosterone secretion can prevent 1ry spermatocytes from progressing to the pachytene stage of the 1st meiotic division *(Chemes et al., 1976).*

3- A decrease in follicular stimulating hormone (FSH) secretion leads to a delayed maturation of spermatozoa *(Brown-Grant et al., 1975).*

Belido et al. (1990) mentioned that, neonatal rats given estrogen at the 1st day of life, with administration of FSH and lutenizing hormone (LH) during the early postnatal period (1-10 days) abolished the effects of estradiol on the morphological and functional development of the testes but does not alter the effects of estradiol on the development of the sex accessory glands. These results suggest that neonatal estradiol acts indirectly through an inhibition of gonadotrophin secretion on testicular development and directly on the development of the sex accessory glands.

B- Estrogen also has direct action which causes:

1- Delayed failure of germ cells to proceed beyond the spermatogonia stage *(Steinberger & Duckett, 1965).*

2- Permenant impairment of spermatogenesis *(Ohta & Takasugi, 1974; McLachlan et al., 1975 and Ohta, 1977).*

C- *Estrogen induced cryptorchidism* is responsible for both permenant impairment of spermatogenesis *(Steinberger, 1971)* and progressive loss of germ cells leading to tubules lined only by Sertoli cells *(Kincl et al., 1963 and Bugnon et al., 1973).*

Abdominal temperature in estrogen induced cryptorchidism may prevent normal maturation of the spermatogenesis after recovery or administration of gonadotropins *(Bugnon et al., 1973; Arai et al., 1983)* and may cause irreversible lesions in some tubules *(Steinberger, 1971).*

The biological actions of estrogen on target cells are mediated by two receptors: the estrogen receptor alpha & beta. In male rat the physiological role of estrogen involves multiple effects starting from musculinization of brain areas related to reproductive function and sexual behaviour up to regulation of testicular development and function. Paradoxically, administration of high dose of estrogen during the critical period of neonatal differentiation results in multiple defects in the reproductive tracts that permanently disrupt male fertility *(Tena-Sempere et al., 2000).*

Estradiol-17 beta [E(2)] a natural estrogen in vertebrates is present in the serum and its receptors are expressed in the testis during the whole process of spermatogenesis. To examine the

functions of the female hormone (estrogen) on spermatogenesis in vitro, 10 pg/ml of E (2) was sufficient to induce spermatogonial stem cell division in cultured testicular tissue, therefore confirming the in vivo observations ***(Miura et al., 1999).***

In the present study, we took postnatal days 10, 22, 33, 45 & 60 days for examination of the drug effects (E_2B) on the testis.

Rats at postnatal days 10, 22, 33, 45 and 60 were chosen for this study based on several reasons:

1- The earliest effects on the spermatogenesis are due to the direct action of estrogen reported at postnatal day 10 ***(Ohta & Takasugi, 1974).*** Plasma estrogen levels are high in rats exposed neonatally to E_2B by this age ***(Bellido et al., 1985).***

2- Plasma estrogen levels are normalized in 22-day old rats exposed neonatally to E2B ***(Bellido et al., 1985)*** and therefore permanent spermatogenesis impairment due to a direct action of estrogen may be assessed from day 22 onward. At this age, testes of normal rats reach the scrotum while those of treated rats are still cryptorchid ***(Vitale et al., 1973).***

3- Leydig cells become mature in 33-days old normal rats and secrete testosterone ***(Chemes et al., 1979).*** In estrogenized rats, estrogen lead to abnormalities in maturation of Leydig cells and so the action of estrogen on the spermatogenesis at this age is due to testosterone suppression ***(Brown-Grant et al., 1975).***

4- In 45-day old rats, the spermatogenesis is qualitatively complete in normal rats, so neonatal estrogen exposure on spermatogenesis

has been assessed in 45-days old rat ***(Ohta, 1977)*** when spermatogenesis is already qualitatively complete in normal rats. ***(Russel et al., 1987).*** Testes of estrogenized rats remain cryptorchid until about this age ***(Vitale et al., 1973).***

5- We take the criteria of 60 days old rat as the termination of this experiment as the process of the spermatogenesis becomes fully mature at 60 days old and not before.

Effect of estrogen injection on morphology of the testis:

In this study the testes of treated rats remain intraabdominal, while the testes are normally in the scrotum by the age of 22 days old ***(Vitale et al., 1973).***

In this study, the injected rats showed a decrease in the body weight and the testes regressed in size than normal. This is due to the decrease of cardiac output of the rat and hence blood reaching the testes. This agrees with ***Kapitola et al. (1994),*** who estimated the relative weight and local blood flow in the testes after administration of E_2B as 1 mg per rat or 5 mg per kg subcutaneously in time interval of 3 to 7 days.

Baumgarten et al. (1990) mentioned that, the treatment of neonatal male rats with mifepristone (an estrogen derivative) showed retardation of testicular growth and delayed puberty during adulthood. They added that, testes did not grow beyond 65% that of normal rats.

Histological effects of estrogen on seminiferous tubules:

It was noted that in rats treated with estrogen, that as early as day 10 of age, rete testis was dilated and some tubules nearest to it showed enlarged lumina. This suggests backflow of fluid from the rete testis to the tubules, since at this age, normally tubular fluid secretion begins and initiation of fluid flow occurred from the tubules to the epididymis via the rete testis ***(Russell et al., 1989).***

Male rats exposed to estrogen during intrauterine life show more affection of the rete testis in the form of carcinoma ***(McLachlan et al., 1998).*** Epidemiological findings indicate that both cryptorchid testis and testicular germ cell cancer may be a result of high maternal estrogen levels early in pregnancy. In mice, the migration of the germ cells to the gonadal ridge is completed by the age of 3 days of gestation. Administration of ethinyl estradiol at this critical time leads to initiation of testicular tumors which reaches a significant size by the age of 2 weeks after birth ***(Walker et al., 1990).***

In this study, there was a dilatation of the seminiferous tubules especially those near rete testis in treated rats. Rats treated with low dose estrogen showed less dilatation of the caudal seminiferous tubules. ***Clermont & Huckins (1961)*** said that, this effect may be due to their position further from the rete testis and away from its compressive effect on the tubules.

In this study, impairment of spermatogenesis started from day 22 onward as evidenced by the presence of multinucleated germ cells in a thin epithelium and sloughed into the lumen of the seminiferous tubules. So, the tubules has a much more number of Sertoli cells than the spermatogonia and there are inflammatory cells in the lumen. These findings coincide with that of ***Vitale et al. (1973) and Chemes et al. (1976).***

Intramuscular injection of norethisterone (NET) & Estradiol Benzoate (E2B) once per month resulted in Aspermia in all male monkeys within 60 to 150 days of treatment. However, addition of depot form of testosterone to the regimen restored the sexual behavioral response typical of a normal male but it did not reverse the azospermic state ***(Shetty et al., 1997).***

In the present study, in treated rats at the day 60 of age still there was no descent of the testes into the scrotum. There was permanent impairment of spermatogenesis. The seminiferous tubules were formed only of spermatogenia and Sertoli cells and the appear to be of Sertoli cells was much more than the spermatogonia and no sperms could be detected. These changes in spermatogenesis were present at all parts of the testis (caudal and cranial part) of those treated with high dose E2B. On the other hand, those treated with low dose E2B, the cranial part of the testis showed permanent impairment of spermatogenesis typical to those treated with high dose while the caudal part showed less influence and seemed more or less normal i.e., no dilatation of the lumen, no sloughs, no

inflammatory cells and the series of cells were present till primary spermatocytes only. No spermatids or sperms were observed. This was due to their lying further from rete testis ***(Clermont and Huckins, 1961).*** But, sperms also could not be detected in the seminiferous tubules of treated adult rats. This impairment of spermatogenesis was due to abnormalities of testosterone and gonadotropin secretions ***(Brown-Grant et al., 1975).***

Prenatal and neonatal exposure to natural and synthetic estrogens induces developmental abnormalities in the male reproductive system. These estrogen-induced lesions included cryptorchidism where the testes are arrested intra-abdominal. Testicular tumours and spermatogenic disruption with no developing germ cells are also present and the interstitial cells are organized as a sheath around the dysfunctional tubules ***(Khan et al., 1998).***

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present study was conducted to study the effect of estradiol benzoate injection postnatally on the development of the testis in albino rats. Also, this study tried to enlighten the possible mechanisms by which estrogen can affect the process of spermatogenesis.

In this study, 75 male rats at the 1st day of life were used. These rates were divided into three groups:

Group A: 25 male rats were injected subcutaneously with a single dose of estradiol benzoate (E2B) 0.5 mg/5 gm B.W (low dose).

Group B: 25 male rats were injected subcutaneously with a single dose of E2B 1 mg/ 5gm B.W (high dose).

Group C: 25 male rats were injected subcutaneously with a single dose of normal saline (control group).

5 rats of each groups were killed at postnatal days 10, 22, 33, 45 and 60. The abdomen was opened and location of the testes was detected. Then the testes were removed and preserved in Bouin's solution, then stained by (Hx & E) and examined by light microscope.

It was found that the testes of estrogenized rats remained intraabdominally (while normally the testes reach the scrotum at day 22 old rats) and the size of the testes were smaller than normal.

The seminiferous tubules showed dilated lumena and impairment of spermatogenesis with no sperm production until adult age.

The rete testis showed dilatation and anastomosing of their channels, and there is impairment of spermatogenesis.These changes in spermatogenesis were present at all parts of the testis (caudal and cranial parts) of those treated with high dose E2B. On the other hand, those treated with low dose of E2B, the cranial part of the testis showed permanent impairment of spermatogenesis typical to those treated with high dose while the caudal part showed less influence and seemed more or less normal i.e., no dilatation of the lumen, no sloughs, no inflammatory cells and the series of cells were present till primary spermatocytes only.No spermatids or sperms were observed. This was due to their existence further from rete testis.

As the testicular descent in rat occurred after birth normally and the human testicular descent occurred before birth, the results of this study are typical to that of human pregnant female exposed to estrogen during pregnancy.

In conclusion, our study can support the idea that estrogen exposure to pregnant mothers leads to abnormalities in the male feti reproductive system in the form of cryptorchidism and impairment of spermatogenesis. So we advise to avoid exposure of female to estrogen during pregnancy.

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ARABIC SUMMARY

المخلـــص العـــــربى

تم إجراء الدراسة الحالية لدراسة تأثير هرمون الأستروجين بعد الولادة على نمو الخصية فى الفأر الأبيض وكذلك إلقاء الضوء على الطرق الممكنة التى يمكن أن يؤثر بها هرمون الأستروجين على عملية تخليق الحيوانات المنوية.

استخدام فى هذا البحث ٧٥ فأراً ذكراً تم تقسيمهم إلى ثلاث مجموعات:

مجموعة أ : (٢٥ فأراً) تم حقن الفئران بجرعة ٠,٥ ملجم/٥جم من عقار الأستراديول بنزوات تحت الجلد فى أول يوم من حياتهم.

مجموعـــة ب : (٢٥ فـــأراً) تـــم حقـــن الفئران بجرعة كبيرة ١ ملجم/٥ جم من عقار الأستراديول بنزوات تحت الجلد فى أول يوم من حياتهم.

مجموعة جـــ: (٢٥ فأراً) المجموعة الضابطة تم حقن الفئران بمحلول ملح تحت الجلد فى أول يوم من حياتهم.

ثـــم أخذ خمسة فئران من مجموعة (أ) و (ب) و (ج) وتم قتلهم عند أيام ١٠ ، ٢٢ ، ٣٣ ، ٤٥ ، ٦٠ يوم. ثم فتحت بطونهم وتم التعرف على الخصيتين وتم نزعهم ثـــم حفظـــوا فى محلول حافظ وتم تجهيز العينات وفحصها بالميكرسكوب الضوئى بعد صبغهم بصبغة الهيماتوكسلين والأيوسين.

ولـــو حظ أن الخصيتين فى الفئران المحقونة تظلان البطن ولا تصلا إلى كيس الخصية بينما تنزل الخصية الطبيعية إلى كيس الخصية ابتداء من اليوم ٢٢ كما أن حجم الخصية فى الفئران المحقونة أصغر من مثيلاتها فى المجموعة الضابطة.

ولقـــد أظهرت الشبكة الخصوية تضخماً وتشابك فى أجزاؤها كما لوحظ وجود فرق فى التأثير على أجزاء الخصية. فالجزء من الخصية القريب من الشبكة الخصوية يكـــون التأثـــير مشابه لما هو فى مجموعة الجرعة العالية. أما ذلك الجزء البعيد عن الشبكة الخصوية فالتأثير يكون أقل.

مـــن الملاحـــظ أن نزول الخصية فى الفئران يحدث بعد الولادة أما فى الإنسان فيحدث قبل الولادة لذلك تأثير الأستروجين على الفئران بعد الولادة هو نفس تأثيره على الإنسان قبل الولادة عند تعرض الأم الحامل لأستروجين.

فى النهاية توضح هذه الدراسة أن تعرض الأمهات الحوامل للأستروجين يؤدى إلى تغيرات فى الأجنة الذكور وذلك فى صورة عدم نزول الخصيتين إلى كيس الخصية وعدم تكوين الحيوانات المنوية. لذلك ينصح بعدم تعرض الأم الحامل للأستروجين.

تأثيـــــر حقن عقار إستراديول بنزوات بعد الولادة على نمو الخصية فى الفأر الأبيض

رسالة مقدمة من

الطبيبة/ ساميـــة محمـــود منـــاوى

بكالوريوس الطب والجراحة

توطئة للحصول على درجة الماجستير فى التشريح

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كليـــة طـــب بنهـــا

جـــامعـــة الـــزقازيـــق

٢٠٠١