



Effect of Estradiol Benzoate on Epididymis Duct in Male Wistar Rats at Pre-Puberty

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Abstract | Male reproductive organs are permanently changed in rodents exposed to high quantities of natural estrogens early in life; however, it is still unclear if exotic estrogens have the same adverse effects. The objective of this study was to investigate the effect of estradiol benzoate (50 µg/rat) on the epididymis in male Wistar rats pre-puberty (aged 50 days). Ten male rats, aged 35 days, were divided equally into control (G1) and treatment (G2) groups. Rats in control were subcutaneously administered 0.25 ml of olive oil; treated rats received subcutaneous injections of 50 µg/rat of estradiol benzoate dissolved in 0.25 ml of olive oil for 15 successive days. After 24hrs from the last treatment session, rats in both groups were weighed, put under general anesthesia, and blood samples were taken to estimate testosterone and estrogen serum concentrations using competitive ELISA kits. The scrotum was dissected, the epididymis detached from the testis, and the weight and length were taken, then divided into three divisions (caput, corpus, and cauda) for histopathological study. HandE staining was used to detect general structures and histopathological changes. Special stains are used to detect collagen fibers, and mucopolysaccharide. The treated group (G2) shows a significant reduction in total length of epididymis and segments, as well as a non-significant drop in total weight. Histologically, the epididymis tubules showed a significant decrease in diameter; the lumen was empty of sperm; there was an increase in epithelial height; hyperplastic epithelia; and an appearance of cribriform forms with infiltration of inflammatory cells. Also, treated rats in G2 exhibited significantly ($P \leq 0.05$) lower blood testosterone and higher estradiol levels. This study concluded that rats exposed to 50 µg/rat of estradiol benzoate pre-puberty showed epididymis toxicity effects.

Keywords | Epididymis toxicity, Estradiol benzoate, Puberty, Pre-puberty, Male infertility, Sperms viability

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INTRODUCTION

The epididymis duct of mammals joins the efferent ducts to the vas deferens. It links the testis cranial pole

via the tunica of the caput and the epididymis testicular connective tissue (Chen *et al.*, 2021; Tavares, 2022). The epididymis consist of of the caput, corpus, and cauda segments. In rodent species, just one common duct coils near

the initial segment after entering the epididymal capsule, however in humans, the head (caput) is predominantly constituted of efferent ducts (Hess *et al.*, 2021).

The epididymal duct in rats is further subdivided into 19 sub-segments; sub-segments 1-4 are associated with the "initial segment" area, while sub-segments 5-11, 12-13, and 14-19 are associated with the caput, corpus, and cauda, respectively. During the postnatal period, the epididymis duct line with pseudostratified columnar epithelial tissue including a range of cell types, containing principal, basal, apical, clear, narrow, and halo cells. Additionally, each segment's epithelial cells are surrounded to varying degrees by dendritic cells and smooth muscle fibers (Knoblauch *et al.*, 2018; Avellar and Hinton, 2019). The amount and appearance of distinct cell types vary amongst the epididymis segments. Each segment contributes differently to the luminal location, which is necessary for testicular sperm to grow and reach the cauda. Ions, water, and small organic compounds are resorption in the initial segment by principal cells. In the caput the principal cells secrete proteins (Marchiani *et al.*, 2017; James *et al.*, 2020) and in the cauda the principal cells have supra-nuclear area contains lipids. A part of the lipid and protein components of the sperm plasma membrane may be altered by these cells in the corpus and caput. Other cell kinds include halo (immune function) cells, clear (large, elevated cells with absorptive function), and basal (small, peripheral cells with de-toxicant activity). Compared to the caput/corpus segments, the cauda's epithelium is shorter and clear cells that phagocytose cytoplasmic droplets and other luminal waste (De Grava Kempinas and Klinefelter, 2014; Whitney and Suttie, 2018). Sperm concentration, maturation (including sperm motility acquisition and fertilizing ability), storage, and protection are all accomplished by the epididymis, an important reproductive organ. While traveling through the epididymis, the immature testicular spermatozoa develop motility and fertilization abilities. When sperm cells interact with the distinct luminal environment that each epididymal region's cells create, sperm maturation takes place during epididymal transit (James *et al.*, 2020).

Male fertility depends mostly on androgen receptor (AR) signaling for the formation of complete spermatogenesis in the testis (Cornwall, 2009; O'Hara *et al.*, 2011; Cooke *et al.*, 2017). Also, circulation androgens and lumicrine hormones derivative from the testis sustenance the development and conservation of a fully differentiated and functional epididymal epithelium in mammals, especially in the initial segment in rodents, like the principal cells' preservation of shape during apoptosis are observed (Ezer and Robaire, 2002; Dacheux and Dacheux, 2014). Epididymal segments have luminal conditions influenced by hormones, primarily androgens and estrogens E2, which aid in sperm maturation and sperm viability. Testicular fluid

travels via the duct and vasculature, feeding the epididymis with testosterone (Marchiani *et al.*, 2017; Dewaele *et al.*, 2022). Both estrogen and androgen have the capability to regulator the expression of genes unique to a particular area and the multiplying of epithelial cells (Hess *et al.*, 2021).

The role of estrogens in regulating testicular cell function and reproductive events is supposedly due to the wide expression of two nuclear estrogen receptors (ERs), ER-alpha and ER-beta and a trans-membrane G protein-coupled estrogen receptor (GPER) in the testis (Liguori *et al.*, 2024). Males may have high estrogen levels in semen and rete testis fluid, in compare to serum estrogen of females, the testis contains estrogen receptors alpha and beta, regulating prolactin, testosterone, and gonadotropin hormones through negative feedback (Dumasia *et al.*, 2015). Reduced estrogen levels in mutant men effect sperm motility and increase the frequency of sperm morphological defects (Dostalova *et al.*, 2017; Marchiani *et al.*, 2017; Cooke and Walker, 2022; Dewaele *et al.*, 2022). Oestrogen, which is produced when testosterone (T) is aromatized (Srilatha and Adaikan, 2011). Most germ cells, Sertoli cells, and Leydig cells locally create E2 in the adult testis through the conversion of testosterone to E2 by the cytochrome P450 enzyme aromatase, which starts early in the gestational cycle and significantly increases it later. External genitalia, the prostate, and adipose tissue are other cells that make estrogens (Lazari *et al.*, 2009; Dostalova *et al.*, 2017; Cunha and Baskin, 2021). The cytochrome P450 aromatase, which is likewise expressed in response to spatiotemporal control, maintains this equilibrium (Li *et al.*, 2015; Hess *et al.*, 2021; Cunha and Baskin, 2021). The primary biological agents of nuclear estrogen receptors (ER α and ER β) are the germ and somatic cells in the test; therefore are the distinct roles that ERs and AR play in controlling the sex steroid receptors during spermatogenesis (Kumar *et al.*, 2018) Since both ER α and ER β are widely localized along with the epithelial cells of the epididymis especially in the epididymal cauda (Gur and Aktas, 2020), estrogen may be involved in regulating epididymal function, especially at the latency time through altering epididymal contractility during the emission phase of ejaculation (Chen *et al.*, 2020)

According to Fisher *et al.* (1997); Cooke *et al.* (2017); Hess *et al.* (2021) the connective tissue nearby the tubules in the caput is less likely to produce ER β than the testis and epididymis of newborn rats. Both the initial segment of the epididymis and epithelial cells of efferent ductules are associated with ER α .

Oestrogen-induced spermatogenesis involves germ cell survival, apoptosis, spermatid proliferation, differentiation, and maturation (Careau and Hess, 2010). Although it's exact role in the epididymis is uncertain. It regulates secretory activities in the epididymis epithelium (Gur and Aktas,

2020), and fluid reabsorption by increasing sperm concentration the efferent ductule prior to the sperm entering the epididymis (Davis and Pearl, 2019; Menad *et al.*, 2021). Significant amounts of 17 β -estradiol have been found in the lumina of the efferent ducts, the caput and cauda of epididymal rats, and the intratesticular fluid (Cunha and Baskin, 2021; Tavares, 2022).

The assessment of male reproductive pathology is becoming increasingly essential in the toxicology of drugs. The study of endocrine disruptors has impacted the analysis of environmental toxicants, and determining any adverse special effects on male reproduction is a crucial part of assessing the safety of medications (Whitney and Suttie, 2018; Carreau and Hess, 2010). Men's estrogen supplementation is relatively uncommon, despite its use in treating prostate cancer (Ockrim *et al.*, 2003), men's memory loss (Beer Tomasz *et al.*, 2006), male vasomotor symptoms, cardiovascular toxicity, improving quality of life, and treating schizophrenia in men (Kulkarni *et al.*, 2013). Exposure to environmental estrogen-like chemicals, also known as endocrine disruptive compounds (EDCs), has been linked to male reproductive tract disorders, which are linked to reduced semen quality and impaired fertility in men. These disorders are explained by testicular atrophy and the slow development of the testis and epididymis (Li *et al.*, 2015). For these reasons, the purpose of this research is to determine whether the exotic usage of estradiol benzoate has an adverse effect on the epididymal structures of immature male rats before the age of fifty days.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Ten mature male Wistar rats, weighing 155.7 $\pm$ 9.21gm, at 35 days of age (pre-pubertal age), were acquired from the Animal House at the University of Qadisiyah's Faculty of Veterinary Medicine. The rats were housed in an air-conditioned room in a laboratory setting. Keeping a constant temperature of 23 $\pm$ 2°C and relative humidity of 66-71% while utilizing 12-hour light and dark cycles. Each was put separately in 47 x 34 x 18 cm plastic cages that were lined with wood chips (Favareto and Fernandez, 2011). The cages were made in England. The rat was provided with unlimited access to water and pelleted standardized food (commercial mouse chow) fifteen days prior the experiment for accommodation to ensure their comfort (D'Souza, 2003; Ilbey *et al.*, 2009). In order to prevent any physiological or biological alterations in the organs and tissue of the rats, only healthy rats were used in this investigation. The rats in both groups were weighted before and after the treatment.

EXPERIMENTAL DESIGN AND TREATMENT

Ten adult male Wistar rats aged 35 days (pre-pubertal age) were randomly divided into two groups, (n=5) of each

group. In randomized double-blind study; rats in the first group (G1) (non-treated group), received a single subcutaneous injection of 0.25 ml of pure olive oil (sham hormone injection) on the dorsum of the animal for fifteen days (Atanassova *et al.*, 2005). Rats in the second group (G2) (Treated group), received a single subcutaneous injection on the dorsum of the animal containing 50 μ g/rat of estradiol benzoate (vetastrol, Iran) dissolved in 0.25 ml of olive oil for fifteen days. The dose was chosen in light of studies conducted by Singh *et al.* (1970); Rau and Chinoy (1984); Kaushik *et al.* (2010).

HORMONE ANALYSIS

After general anesthesia by IM injection of a mixture of Xylazine (10mg/kg BW), and Ketamine (80mg/kg BW) (Opeyemi *et al.*, 2021). In order to acquire serum for hormonal investigation, blood samples were collected from the left ventricle of the heart and put in tubes containing ethylene diamine tetraacetic acid (EDTA) (BD Vacutainer, USA) as the anticoagulant (Brouard *et al.*, 2016), centrifugation at 3000 rpm for 15 minutes was used to separate the serum then was drawn and stored at -20°C for measurement levels of testosterone (T) and 17 β -oestradiol (E2) (Brouard *et al.*, 2016). Serum levels of testosterone and 17 β -oestradiol were determined using commercial competitive ELISA kits (Rat Estradiol ELISA Kit Cat. NO EA0011Ra), (Rat Testosterone ELISA Kit Cat. NO EA0023Ra) from Bioassay Technology Laboratory (BT LAB, China) (Hammodi *et al.*, 2011; Kazemi *et al.*, 2016).

MORPHOLOGICAL ANALYSIS

Twenty-four hours following the last treatment, in the early afternoon, the tissues (epididymis) from each animal were collected (Rau and Chinoy, 1984). Under general anesthesia, longitudinal skin incisions were made in the scrotal wall to remove the testes from scrotum, separate and remove the epididymis from the testis. Total length and weight of each epididymis (right and left) were measured by venire caliper and weight sensitive balance. The anatomical form and location of the caput, corpus, and cauda were identified, and measure the length and weight of each epididymis segment (Figure 1) (Olukole *et al.*, 2009). The relative organ weight were determine using the following formula: Relative organ weight (%) = Organ weight (g)/ Body weight (g) x 100 (Brouard *et al.*, 2016).

HISTOLOGICAL STUDY

In order to clean the epididymis, 0.9% NaCL saline solution was used as usual. Following the extraction of any residual fat, the caput, corpus, and cauda samples were preserved for 28 hours in 10% neutral buffered formalin (NBF). Samples were submerged in 70% ethanol for a full day following their washing with tap water. Next, they proceeded with the standard procedures for histological preparation, which comprise paraffin embedding, clearing, impregnation, and

dehydration. Following paraffin sections (5 μ m) of these organs. Generally, histological structures are highlighted with the Harris Hematoxylin and Eosin (HandE) stain and use specific stains such as Masson trichrome, Van Gieson's and Wiegert's elastic stain, for collagen fibers investigation. Periodic Acid-Schiff (PAS) staining was also used to demonstrate mucopolysaccharides (Luna, 1968). Twenty-five tubules of five slides from each specimen of each rat were evaluated quantitatively by light microscope (Olympus, Japan). An ocular micrometer magnified 40 X was used to assess the duct's tubule diameter and epithelium height (Ilbey *et al.*, 2009; Al-Allaf and Al-Ashoo, 2021).

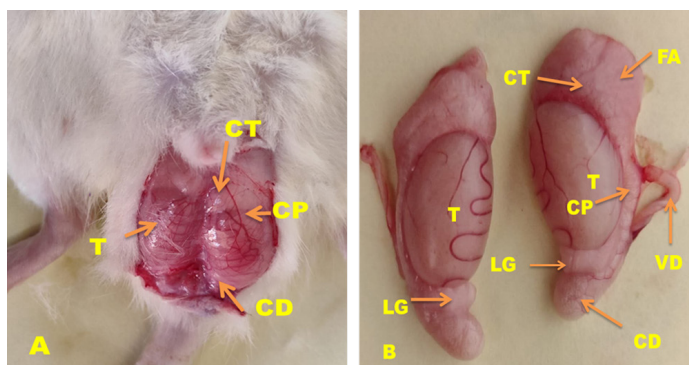


Figure 1: (A) Ventral view of mature wistar rats aged 66 days showed male genital system (B) Showed epididymis duct lie along dorsal surface of testis (T) and epididymal segments: caput (CT), corpus (CP), cauda (CD) that connect with testes by ligament (LG) and continuous with vas deferens (VD).

STATISTICAL ANALYSIS

SPSS® software version 24 was used to analyze the data. Using the one-way analysis of variance and the post Hoc analysis, two groups were compared as Means $\pm$ Standard Errors throughout all segments of the epididymal rats. Value was measured as significant at $P < 0.05$. A post hoc multiple comparison test was used to compare the treatment groups against the control group. To compare medians, an ANOVA was used, and then a post hoc multiple comparison test was performed. A significance threshold of 0.05 was applied to probability values. All values, unless otherwise indicated, are given as mean $\pm$ SE (M $\pm$ SE) (Reddy, 2019; Jabori *et al.*, 2023).

RESULTS AND DISCUSSION

MACROSCOPIC STUDIES

The epididymis, at the 51st day of age in both groups appears as an elongated, crescent-shaped tubular duct, is located inside the scrotum along the testis's dorsolateral surface. It consists of three components: caput, corpus, and cauda, covered in fat. The caput epididymis is convex and surrounded by fat pad, while the corpus epididymis is horizontally dorsal and has a pointed end (Figure 1).

MORPHOLOGICAL CHANGES

At the end of treatment period, the body weights of G1 and G2 were increased slightly (165 ± 6.59 ; 183 ± 7.81 gm.), in compared to their initial weights (155.8 ± 9.67 ; 155.6 ± 9.86 gm.), respectively. However, the relative weight of G2 was significantly ($p < 0.05$) lower (0.08 ± 0.02 gm.) in compared to the relative weight of G1 (Table 1). The epididymal segments of G2 displayed atrophy in the form of the epididymis duct that seemed rougher and smaller on the outside than that of G1 (Figure 1, 2).

Table 1: Effect of (50 μ g/rat of estradiol benzoate) treatment pre- puberty on body weight (BW) and relative weight (mg/100 g BW) of epididymis at puberty (66 day) in Wistar male rats. non-treated group (G1) and treated group (G2).

Groups	Body weight (BW)	Relative weight of epididymis (mg/100 g BW)
G1	183 ± 7.81	1.23 ± 0.15
G2	165 ± 6.59	$0.08 \pm 0.02^*$

The value* was estimated significant at $P < 0.05$.

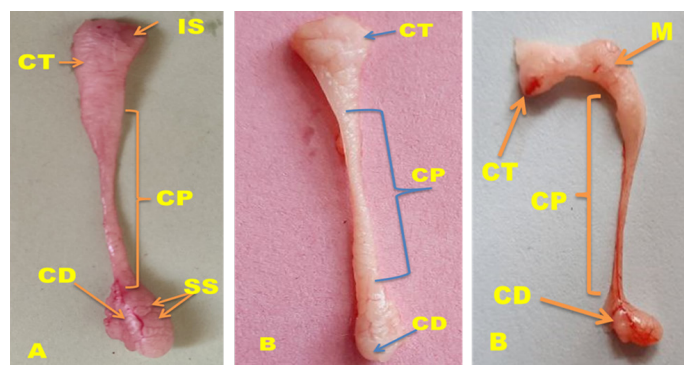


Figure 2: Gross anatomy of the epididymis segments: caput (CT), corpus (CP), cauda (CD) of wistar Rats aged 66 days that split into lobules or sub-segmental subdivision (SS). (A) Epididymis duct non-treated group (G1). (B) Epididymis duct in treated group (G2) with 50 μ g/rat of estradiol benzoate that appeared irregular in shape as malformed (M) (right picture).

MORPHOMETRIC

The weights of the right epididymis in G1 and G2 were different than the left (0.22 ± 0.09 ; 0.21 ± 0.07 gm.), (0.15 ± 0.01 ; 0.13 ± 0.03 gm.) respectively. The weights of epididymis segments (caput, corpus, and cauda) in G1 of right side were (0.08 ± 0.002 ; 0.06 ± 0.003 ; 0.07 ± 0.006 gm.) respectively and in left side were (0.08 ± 0.005 ; 0.06 ± 0.002 ; 0.067 ± 0.002 gm.) respectively, while in G2 the weights of right side were (0.06 ± 0.005 ; 0.04 ± 0.008 ; 0.03 ± 0.004 gm.) respectively and in left side were (0.06 ± 0.006 ; 0.04 ± 0.005 ; 0.048 ± 0.007 gm.) respectively. The weights of epididymis duct and epididymis segments in G2 were decreased than weights in G1 but without significant ($P < 0.05$) difference (Table 2, 3).

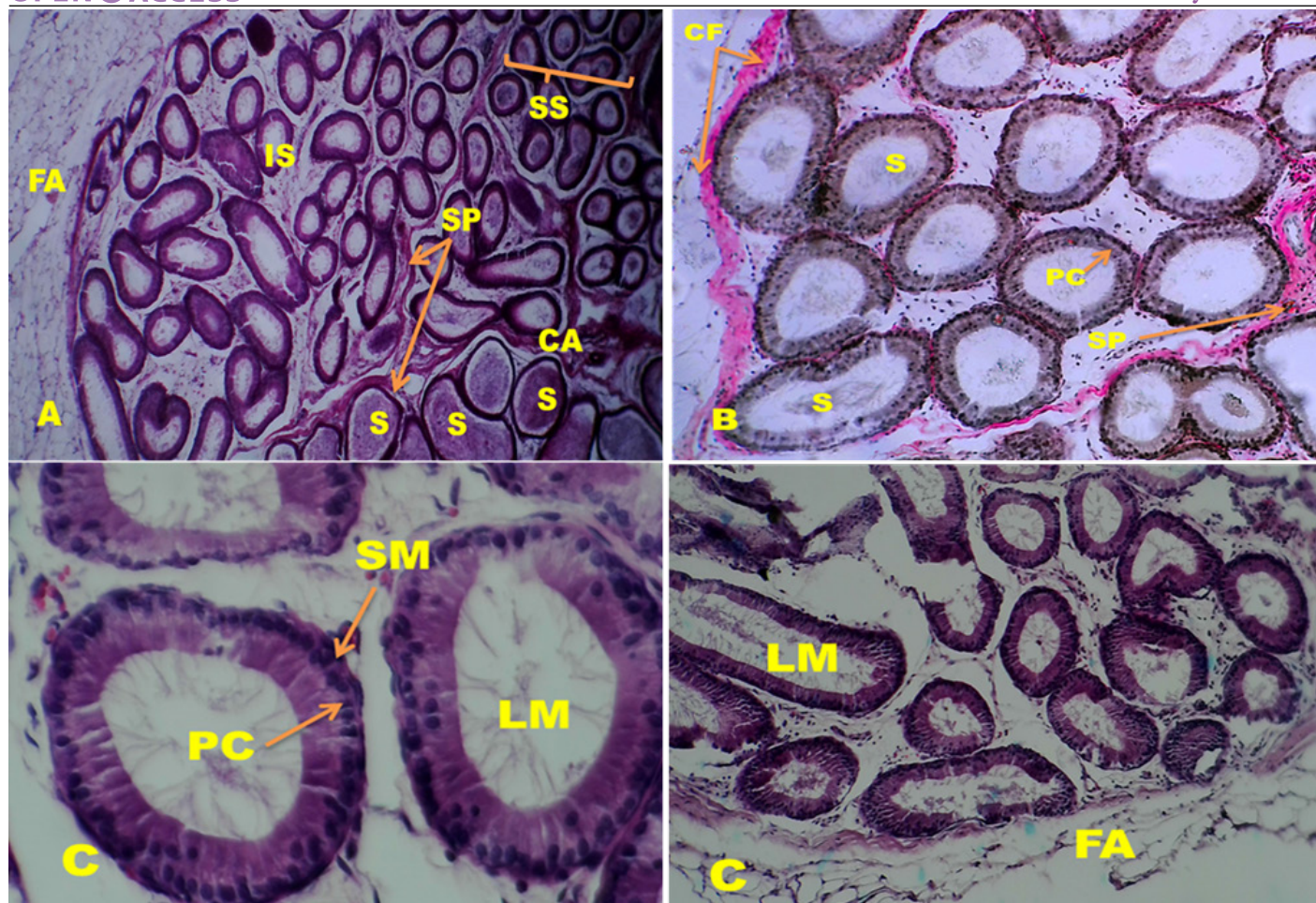


Figure 3: Microscopic section of epididymis segments (A) Initial (IS) and caput segments (CA) of Wistar Rats aged 66 days in non-treated groups showed were surrounded by friable amount of fat (FA) and divided into sub-segmental division (SS), by loose connective tissue septa (SP) rich with collagen fibers (CF). Principle cells (PC) one type of epithelial cells lined tubules that full with sperms. (A) HandE 40X; (B) Initial (IS), of non-treated groups (G1) showed ducts surrounded by clear quantity of collagen fiber (CF) and ducts divided by loose connective tissue septa (SP), each ducts full with sperm (S) Van Gieson's stain 100X (C) Initial segments of treated groups (G2) showed lumen duct (LM) devoid of sperm, each tubule surrounded from out by layers of smooth muscle (SM), and lined by epithelia that contain most cell such as principle cell (PC). (C) HandE 400X (left picture), 100X (right picture).

Table 2: Displayed the total weight (gm) and length (mm) of the left and right epididymis of Wistar Rats aged 66 days in the non-treated group (G1) and treated group (G2) with 50µg/rat of estradiol benzoate. Mean ± standard error.

Groups	Total length (R) (mm)	Total length (L) (mm)	Total weight (R) (gm)	Total weight (L) (gm)
G1	42.32±0.85*	40.10±1.03*	0.22±0.009	0.21±0.07
G2	35.85±0.93	35.85±0.99	0.15±0.01	0.13±0.03

*The value was deemed significant at $P < 0.05$.

The total epididymis duct's length reduced in G2 when compared with G1. The results show a statistically significant difference at $P < 0.05$ between the right and left total length epididymis of G1 (42.32±0.85, 40.10±1.03 mm) and G2 (35.85±0.93, 35.85±0.99) respectively (Table 2). The length of right epididymal segments (caput, corpus, and cauda) in G1 was (8.57±0.53; 25.65±0.36; 8.09±0.41 mm) respectively showed statistically significantly different from

those of G2 (7.1±0.32; 20.37±0.06; 7.87±0.32 mm) respectively. The lengths of left epididymal segments (caput, corpus, and cauda) in G1 (8.42±0.35; 24.12±0.62; 7.81±0.23 mm), and in G2 (7.40±0.38; 21.34±0.85; 6.86±0.32 mm) respectively. There were significant differences ($P < 0.05$) in the length of left corpus only (Table 3).

HISTOLOGICAL STUDIES

The epididymis segments found lined with pseudostratified columnar epithelial tissue made of several cell types; the principal of long stereocilia, apical, basal, and clear cells. A layer of smooth muscle fibers encircled the lumen of the epididymal tubules, which was filled with sperm. Additionally, each of the epididymal segments was separated into sub-segmental sub-divisions by loose connective tissue that rich with collagen fibers and some elastic fibers. The epithelial lining was appeared positive reaction for PAS stain (Figure 3A, 4A, 5A, 6A).

Table 3: Displayed the weight (gm) length (mm) of the left and right epididymal segments (caput, corpus, and cauda) of Wistar Rats aged 66 days in the two groups: the non-treated group (G1) and treated group (G2) with 50µg/rat of estradiol benzoate with 50µg/rat of estradiol benzoate. Mean ± standard error.

Group / sample	Length Right	Length Left	Weight(R)	Weight(L)
G1 Caput	8.57±0.53*	8.42±0.35	0.08±0.002	0.08±0.005
Corpus	25.65±0.36*	24.12±0.62*	0.06±0.003	0.06±0.002
Cauda	8.09±0.41*	7.81±0.23	0.07±0.006	0.067±0.002
G2 Caput	7.1±0.32	7.40±0.38	0.06±0.005	0.06±0.006
Corpus	20.37±0.06	21.34±0.85	0.04±0.008	0.04±0.005
Cauda	7.87±0.32	6.86±0.32	0.03±0.004	0.048±0.007

The value* was estimated significant at P<0.05.

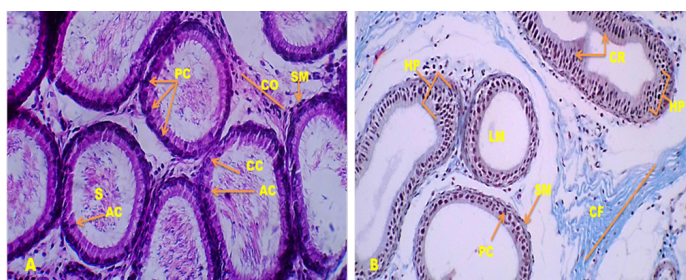


Figure 4: Microscopic section of caput of epididymis of Wistar Rats aged 66 days. (A) Caput of epididymis in non-treated group (G1) showed lumen (LM) full with sperms (SP), and lined with pseudostratified columnar epithelial that have apical cells (AC), principle cells (PC), clear cells (CC). The tubules surrounded by layers of smooth fibers (SM) and separated by loose connective tissue (CO). HandE, 200X. (B) Caput of epididymis of treated groups (G2) with 50µg/rat of estradiol benzoate, showed lumens (LM) devoid of sperms. There was hyperplasia (HP) and increased in thickness of epithelial and appeared cribriform forms (CR). There was a mount of collagen fibers (CF). Masson trichrome stain, 200X.

HISTOPATHOLOGY CHANGES OF THE EPIDIDYMIS

The study showed histological alterations in all epididymis segments in G2, such as; degeneration of most epithelial cells, bubbly lipid vacuolization in the supra-nuclear area of the principal cells which are frequently seen in the cauda or distal corpus (Figure 5B, 6B), defeat in clear cells in cauda segment (Figure 6B), Hyperplastic epithelium was seen lead to increase in thickness of epithelial lining and appeared as cribriform forms or enfolding of the epithelium in all segments except initial segment. There were increased in collagen fibers in the septa between segments with inflammatory infiltrates (Figure 4B, 5B, 6B). The lumen of tubules of all epididymis segments was smaller than that of G1. The lumen appears irregular or shrinking in most epididymis segments. Most lumens were appeared empty of sperms and debris tissue found in other lumens, and

rupture of the epididymal tubule especially in the cauda segment also seen (Figure 6B). These finding differ when in comparison to that of G1 (Figure 3A, 4A, 5A, 6A).

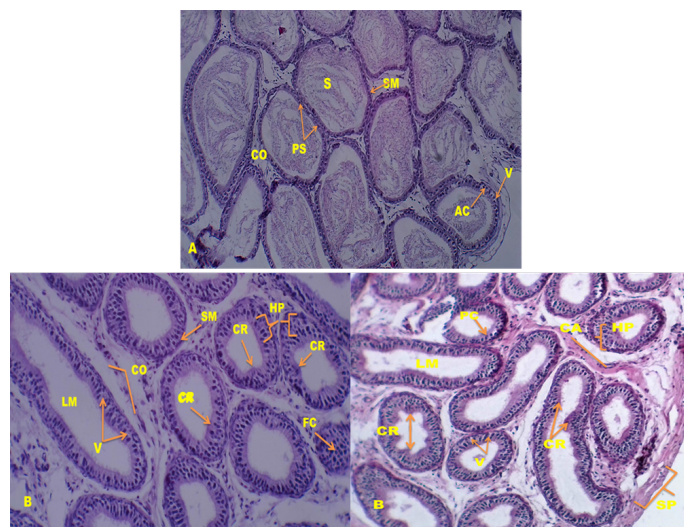


Figure 5: Transverses section of corpus of epididymis of Wistar Rats aged 66 days. (A) corpus in non-treated group (G1) showed tubulars lined with pseudostratified columnar epithelial that consist of principle cell (PC) and it was full with sperms (S). HandE, 100X (B) Corpus of epididymis of treated groups (G2) with 50µg/rat of estradiol benzoate, showed tubules separated by loose connective tissue (CO) as septa (SP) that rich with of collagen fibers (CA) and surrounded by smooth muscle fibers (SM). Tubular lumen (LM) devoid of sperms and there was hyperplasia (HP) with increased in thickness of epithelial and appeared cribriform forms (CR), and increase of bubbly lipid vacuolization (V) in the supra-nuclear area of the principal cells and inflammatory infiltrate (FC). (B) Left: HandE, 200X; Right PAS Stain 100X.

HISTOMORPHOMETRIC ASSESSMENT

The mean epithelial height of initial and cauda segments in G1 was (26.87±1.70, 19.11±0.77 µm) respectively, show high as compared with that in caput and corpus segments (14.38±0.49; 18.37±0.76 µm), respectively, but without significant p≤0.05 difference. The initial, caput, corpus, and cauda epididymal segments of the rats in G2 appeared to have mean epithelial heights of (35.40±1.87, 33.37±4.71, 32.12±5.33; 33.04±0.00 µm) respectively, which was higher than those of G1 (26.87±1.70; 14.38±0.49; 18.37±0.76; 19.11±0.77 µm) respectively; however, no significant p≥0.05 difference was observed (Table 4).

The mean tubular diameter of G1 increased toward the caudal direction, with the initial segment's tubular diameter being the smallest and the cauda segment's tubular diameter being the largest in relation to the other segments. The mean tubular diameter measurements for every segments in G1 were (90.54±3.27; 186.06±4.95; 195.24±9.28; 221.36±14.13 µm) respectively. There were distinguished

variations observed across all epididymis segments at the $p \leq 0.05$ level. The mean tubular diameters of the caput, corpus, and cauda of the rat epididymal in G2 were (117.75 ± 17.39 ; 79.39 ± 3.70 ; 76.57 ± 23.33 μm) respectively, which was smaller than those of G1, with a significant difference at the $p \leq 0.05$ level. The tubular mean initial segment diameter in G2 was found (79.39 ± 3.70 μm) smaller than that of G1, but there was no significant $p \leq 0.05$ difference (Table 4).

Table 4: Histological dimension of the epididymal segments of Wistar Rats aged 66 days in non-treated (G1) and treated groups (G2) with 50 μg /rat of estradiol benzoate. TD=tubular diameter, EH=epithelial height. Mean $\pm$ SE (μm).

epididymis segments	G1		G2	
	TD μm	HE μm	TD μm	HE μm
Initial	90.54 $\pm$ 3.27	26.87 $\pm$ 1.70	79.39 $\pm$ 3.70	35.40 $\pm$ 1.87
Caput	186.06 $\pm$ 4.95*	14.38 $\pm$ 0.49	117.75 $\pm$ 17.39	33.37 $\pm$ 4.71
Corpus	195.24 $\pm$ 9.28*	18.37 $\pm$ 0.76	79.39 $\pm$ 3.70	32.12 $\pm$ 5.33
Cauda	221.36 $\pm$ 14.13*	19.11 $\pm$ 0.77	76.57 $\pm$ 23.33	33.04 $\pm$ 0.00

*The value was estimated significant at $P < 0.05$.

HORMONAL ASSESSMENT

Table 5, showed a significant ($p \leq 0.05$) increase in estradiol concentration in the treated group (G2) with 50 μg /rat of estradiol benzoate (865.44 $\pm$ 61.92 ng/ml) compared to the non-treated group (G1) (616.72 $\pm$ 143.36 ng/ml). However, testosterone concentration (2.64 $\pm$ 0.26 ng/ml) decreased in the treated groups (G2) but did not differ significantly from the non-treated group (G1) (2.73 $\pm$ 0.26 ng/ml).

Table 5: Hormonal of Wistar Rats aged 66 days in non-treated (G1) and treated groups (G2) with 50 μg /rat of estradiol benzoate. TD=tubular diameter, EH=epithelial height. Mean $\pm$ SE (μm).

Hormones/Groups	Estradiol(E2) ng/ml	Testosterone ng/ml
G1	616.72 $\pm$ 143.36	2.73 $\pm$ 0.26
G2	865.44 $\pm$ 61.92	2.64 $\pm$ 0.26

The value was estimated significant at $P < 0.05$.

Endocrine disrupting chemicals (EDCs), such as exposure to estrogenic or other hormonally active chemical substances (e.g., antiandrogenic), can cause reproductive abnormalities in a developing male fetus. These substances decrease androgen levels and inhibit androgen function, which may be partly or fully responsible for the negative trends in male reproductive health, including the rise in the frequency of male reproductive problems and the decline in sperm counts and quality in humans (Delbès et al., 2009; Cooke et al., 2021; Corpuz-Hilsabeck and Culty, 2023).

The hormones androgen and estrogen, which are present in testicular fluid and the peripheral blood of the epididymis,

control the structures and functions of the epididymis by communicating through a variety of androgen and estrogen receptors (Robaire and Hamzeh, 2011).

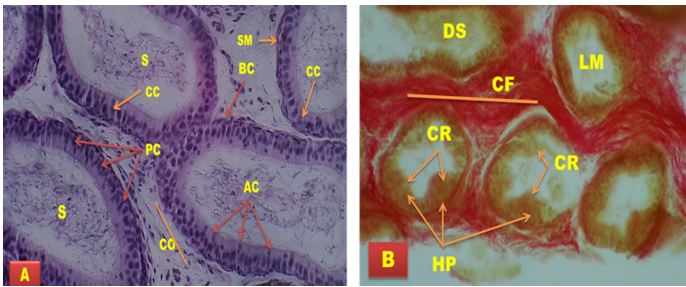


Figure 6: Transverses section of cauda of epididymis of Wistar Rats aged 66 days (A) cauda in non-treated groups (G1) showed pseudostratified columnar epithelial that consist of principle cell (PC), basal cell (BC), lined tubules that full with sperms (S) and between tubules loose connective tissue (CO). HandE, 200X. (B) Cauda of epididymis of treated groups (G2) with 50 μg /rat of estradiol benzoate showed most tubules lumen (LM) irregular in shape, shrinking, smaller and devoid of sperms or had debris (DS). There was hyperplasia (HP) with increased in thickness of epithelial and appeared cribriform forms (CR) and there is large amount of collagen fibers (CF) between tubules. Van Gieson's stains 200X.

The current study found the length and weight of the epididymis as a whole and each of its segments was shorter and lesser in the estradiol-treated group (G2) than that in the control group (G1). Furthermore, the testosterone levels in the treated group decreased following an estradiol injection these findings are consistent with Srilatha and Adaikan (2011), who mention exposure to environmental/herbal estrogens may also cause alteration in hormonal ratio. The study also in agreement with Horan et al. (2017), who demonstrating that excessive exposure to neonatal estrogen can lessen the elongation and coiling of the epididymis as well as the blurring of the epididymal-vas deferens transition. According to Avellar and Hinton (2019), testosterone is essential for the development of the epididymis and adult its function is dependent on epithelial androgen receptors (ARs). Chen et al. (2020) and Cooke and Walker (2022), report that exogenous estrogen an injection cause defects in male reproductive organs and inhibits the hypothalamus-pituitary axis, reducing circulating testosterone.

Epithelial cells of the human and animal epididymis have steroidogenic properties. In vitro, rat epididymal epithelial cells create androgens, which then convert to 17 β -estradiol (Awider-Al-Amawi et al., 2007). The results of this study show that the mean tubular diameters of the treated rats decreased in every segment due to elevated and edematous epithelia, an increase in collagen fiber, and changes in the size and shape of the principal cells, this results are consistent with Hess (2015), and Leavy et al. (2017) who high-

lights the possibility of male reproductive system deformity caused by embryonic exposure to large concentrations of natural estrogens and diethylstilbestrol (DES), since efferent ductules may cause fluid to accumulate in the rete testis and seminiferous tubules led to a significant decrease in seminiferous tubule diameter as well as an increase in collagen founding and fatty degeneration in human testes and eventually testicular atrophy. All of these changes occur due to androgen lack and all of these modifications are constant till androgen ranks improve (De Grava Kempinas and Klinefelter, 2014).

In this study the result showed bubbly lipid vacuolization in the supra-nuclear area of the principal cells are frequently seen in the cauda or distal corpus. The epididymis's metabolism is significantly impacted by absent androgens that lead to the body switches its metabolic energy (ATP) from carbohydrates to lipids (Tavares, 2022).

The results indicate that the cribriform transformation is a hyperplastic modification of the epithelium (which can fold over itself to form pseudo-glandular formations) that occurs in the longest epididymis duct of the treated group of rats. This modification is typically found in the cauda or distal corpus and in some cases, particularly in the cauda, the treated rats also exhibit ruptured epididymal tubules and inflammatory infiltrates. According to De Grava Kempinas and Klinefelter (2014), testicular toxicity in rats can result in epididymis atrophy, altered testicular fluid volume, and disruption of the epididymis microenvironment, therefore disturbances in any of these practices or block of any part of the epididymis duct can lead to sperm immobility, which in turn frequently leads to irritation and inflammatory infiltrates, which are usually neutrophils or lymphocytes, and the rupture of the epididymis tubules got on by elevated intraluminal pressure and it is often related to androgen reduction and by block of adrenergic pathways by α -adrenergic antagonists (Mohammadzadeh *et al.*, 2021; Corpuz-Hilsabeck and Culty, 2023) also agreement with (Pilutin *et al.*, 2021) who mention long-term suppression of aromatase produced same to the present results. In rodents, exposure to exogenous perinatal high estrogen (estradiol) plugs into a paracrine short-loop negative feedback pathway, severely suppressing the generation of testosterone. What this situation does not explain, though, is why excessive estrogen exposure also has a detrimental effect on the production of the androgen receptor protein, thus blocking the action of androgen. This could be interpreted as just another aspect of controlling the ratio of androgen to estrogen action. It may be especially significant in the reproductive system, where it is impossible to negatively feedback control the amount of testosterone available; instead, it regulates the hormone's ability to act through the androgen receptor (Hess *et al.*, 2021). That explain the epididymis changes happened in this study.

The current study leaves open a number of questions, including: (1) Is the effect of exogenous estrogen temporary or persistent over the course of a person's life?; and (2) Do small amounts of pollutants have the same effects in large doses? Future studies on these topics are required.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, according to study findings, the effect of exogenous estrogen (50 micogram estradiol benzoate) on the epididymis of rats before puberty is detrimental and causes epididymis toxicity effects. So, we recommended an immune- histochemical and molecular study to clarify the effect of exogenous estradiol benzoate on the Wistar rat testes at pre-puberty period of age.

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NOVELTY STATEMENTS

This study employed that pre-pubertal rats expose to estradiol benzoate (50 μ g/rat), shown to be harmful to the rats' epididymis tissue. As a result, the rats in this study did not possess sperm when they reached puberty, and preventing them from fertilizing.

AUTHOR'S CONTRIBUTIONS

Eman Faisal Albaghdady: Concept, study plan, statistical evaluation, discussion.

Manar Mousa Alhussein: Materials and Methods and Results.

CONFLICT OF INTEREST

The authors state that there is no conflict of interest with regard to the current study.

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