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## Histological, histochemical and ultrastructural characterizations of the epididymal region of the turkey (*Meleagrisgallopavo*).

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### ABSTRACT

Histological, histochemical and ultrastructural studies were performed on the epididymal regions of ten adult sexually mature apparently healthy male native turkeys (*Meleagrisgallopavo*). The epididymis of turkey was consisted of extra testicular rete, efferent ductules, connecting ductules, and the epididymal duct. The rete testis were lined by squamous to cuboidal cells, while the proximal efferent ductules were lined by pseudo stratified columnar of ciliated, non-ciliated type I and basal cells. The distal efferent ductules were lined by pseudo stratified columnar of ciliated, non-ciliated type II and basal cells. However, the connecting ductules were wide and narrow lined by pseudo stratified columnar of ciliated, non-ciliated type III and occasional basal cells of regular lumen. Narrow connecting ductules consist mainly of ciliated cells while wide consist of non-ciliated. The epididymal duct lined by pseudo stratified non ciliated columnar and basal cells of same size and intensity. Histochemically the reactivity for PAS was detected in the supra nuclear cytoplasm and luminal borders of the efferent ductular epithelium, intraepithelial gland of the epididymal duct and the basement membrane of various ducts and ductules. While such reactivity not seen in the connecting ductules.

**Keywords:** Epididymis, Histology, Histochemical, Ultrastructure, Turkey.

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## INTRODUCTION

Studies on the epididymal region of the turkey had been carried by (Hess et al., 1976; Aireand Josling, 2000 and Zaniboni et al., 2004). This region contained the extra testicular rete testis, ductuli efferentes, connecting ductules, and ductus epididymidis. The histology of the normal epididymal region of the turkey referred to Hess et al., 1976, revealed some maturation of sperm during transportation through the epididymal region. While, this was not crucial for sperm to penetrate and fertilize an ovum (Froman, 1994). In addition the absorption of testicular fluid which increasing in sperm concentration (Zaniboni et al., 2004). Nevertheless, the present study was directed to investigate the histological appearance, histochemical and ultrastructural characteristic features of the epididymal region of the turkey.

## MATERIAL AND METHODS

### General histological study:

Ten adult sexually active apparently healthy male mature native turkeys (*Meleagris gallopavo*) were used in this study. The birds were euthanized (Reilly, 2001) by intraperitoneal injection of sodium pentobarbitone (anaesthetic solution 60mg/ml at dose rate of at least 80mg/kg), the testicles with their epididymis were immediately dissected out and sectioned into small pieces. Some of these specimens were fixed in neutral buffered formalin. They were processed and embedded in paraplast. Serial and step serial sections of 5-6  $\mu\text{m}$  thick were obtained and stained with Haematoxylin and Eosin (H&E), Masson's trichrome. These histological methods were adopted after Bancroft and Gamble (Bancroft and Gamble, 2008).

### Histochemical study:

The epididymal region sections stained with Periodic acid Schiff (PAS) for detection of neutral mucopolysaccharides and glycoproteins and Alcian blue (pH 1, 2.5) for demonstration of acid mucopolysaccharides, Aldehyde fuchsin and Best's carmine. These techniques were used as outlined by Carson (1990).

### Transmission electron microscopy:

Small tissue blocks from the epididymal region were fixed in paraformaldehyde-glutaraldehyde in phosphate buffer (Karnovsky, 1965). Specimens were post fixed in 1 % osmium tetroxide for one hour, washed in 0.1 M phosphate buffer (pH 7.3), then dehydrated in graded ethanol and embedded in open araldite mixture (Mollenhauer, 1964). Semithin sections (1 $\mu\text{m}$ ) were cut, stained with Toluidine blue (Richardson et al., 1960) and examined with light microscope. Ultrathin sections were cut and stained with uranyl acetate and lead citrate. The sections were examined with a JEOL 1010 transmission electron microscope at Regional Center for the Mycology and Biotechnology (RCMB) Al-Azhar University, Cairo, Egypt.

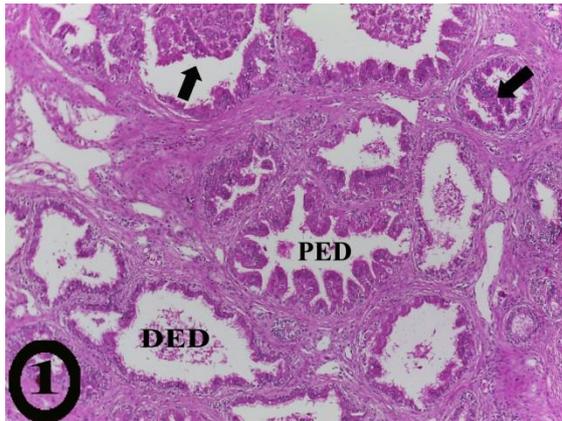
## RESULTS

### Histological and Cytological findings:

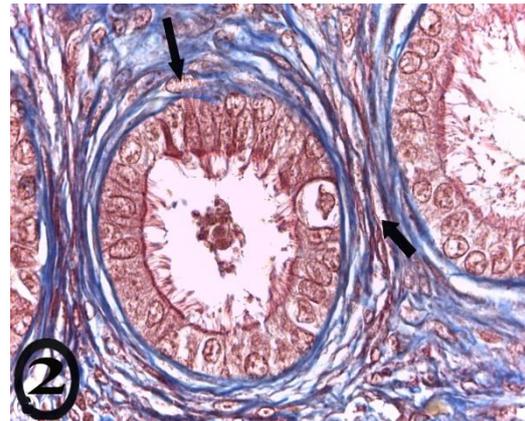
The epididymal region of the turkey consisted of mass of ductules and ducts (Fig.1) which supported by collagenous, smooth muscle fibers (Fig. 2) and reticular fibers. The epididymal region of the turkey consisted of extra testicular rete, proximal efferent ductules, distal efferent ductules, connecting ductules, and the epididymal duct.

### The extra testicular part of the rete testis:

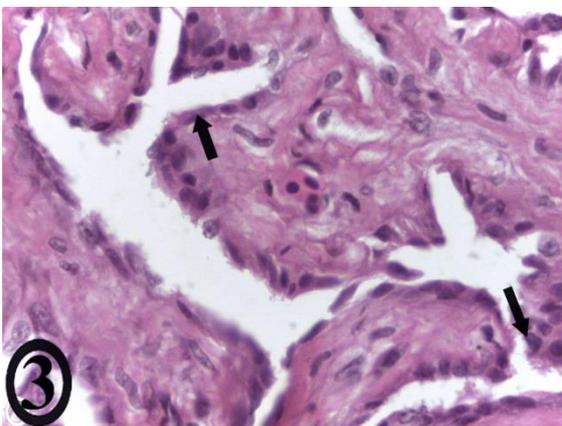
The extra testicular rete testis is a continuation of the intra testicular portion consisted of thin walled channels located just outside the testicular capsule; it is lined by squamous or low cuboidal cells (Fig. 3), which changed into high cuboidal or columnar cells before the beginning of the efferent ductules. The rete testes were supported by highly vascularized connective tissue containing lymphocytic aggregations. Some of these aggregations took the nodular form.



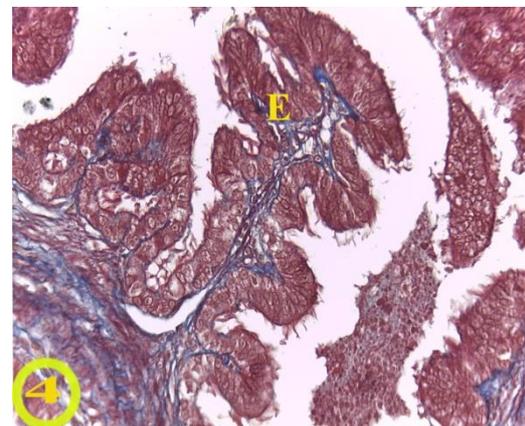
**Fig. (1)** Photomicrograph of a transverse section of the turkey epididymal region, showing proximal efferent ductules (PED), distal efferent ductules (DED) and intra luminal released secretory cells (arrow) H&E X40.



**Fig. (2)** Photomicrograph of a transverse section of the turkey epididymal region, showing periductular collagen and smooth muscles fibers (arrow). Masson's trichrome X1000.



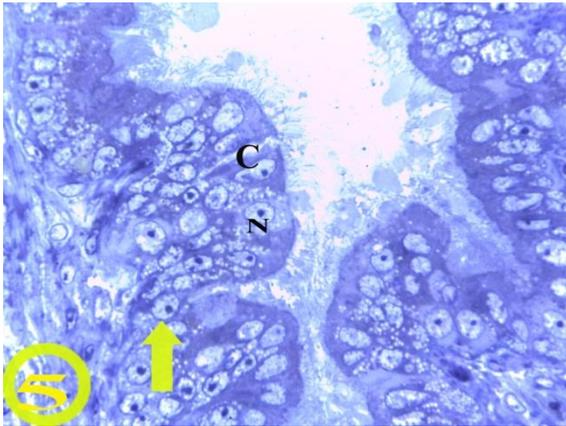
**Fig. (3)** Photomicrograph of a transverse section of the turkey epididymal region, showing extratesticular rete testis lined by squamous or low cuboidal cells (arrow). H&E X1000.



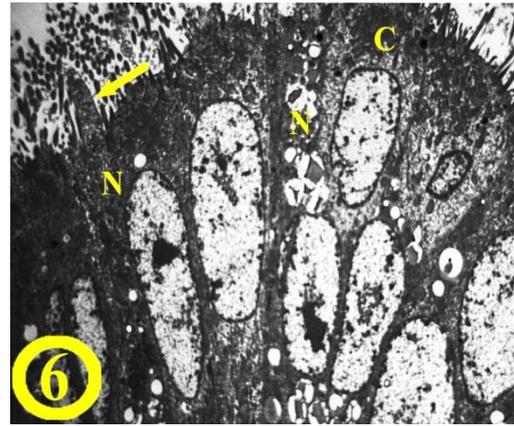
**Fig. (4)** Photomicrograph of a transverse section of the turkey epididymal region, showing proximal efferent ductules (PED) thrown into many longitudinal folds of variable heights (E). Masson's trichrome X1000.

**The proximal efferent ductules:**

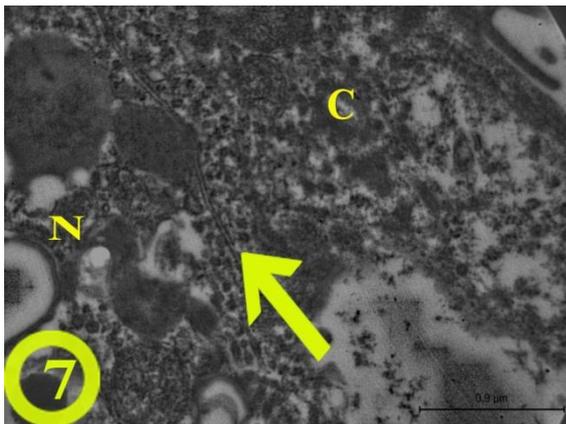
The wall of proximal efferent ductules was thrown into many longitudinal folds of variable heights (Figs.1, 4).The mucosal folds were of leaf to tongue shaped, and having primary and secondary branching and at times tertiary branches. The lumen of the later ductules contained few scattered spermatozoa, immature germ cells, macrophages. The intra luminal released secretory cells have been observed(Fig. 1).Two main cell types were recognized in their epithelial lining, ciliated and non-ciliated cells type I. Additionally, few basal cells with spherical ovoid nuclei (Fig.5).The ciliated cells appeared columnar in shape and had lightly stained cytoplasm and ill-defined cell boundaries. Their nuclei were elongated and lightystained intensity with one or more clear nucleoli. Long tufts of cilia projected it. The non-ciliated cells type I were filled with vacuolated darkly stained cytoplasm with spherical nuclei. Moreover the non-ciliated cells type I might protrude into the lumen to form bleb like projectionswhich, appeared by transmission electron microscope (Fig. 6). There was junction complex between ciliated and non-ciliated (Fig. 7). The non-ciliated cells exhibit numerous tubular coated pits, apical tubules and vacuoles, as well as numerous dense bodies and abundant mitochondria with a concentric arrangement around lipid vesicles and rough endoplasmic reticulum in the supra nuclear zone (Fig. 8). Supra nuclear and infra nuclear cytoplasm of the ciliated cells contained numerous mitochondria, rough endoplasmic reticulum and free ribosomes (Fig. 9).



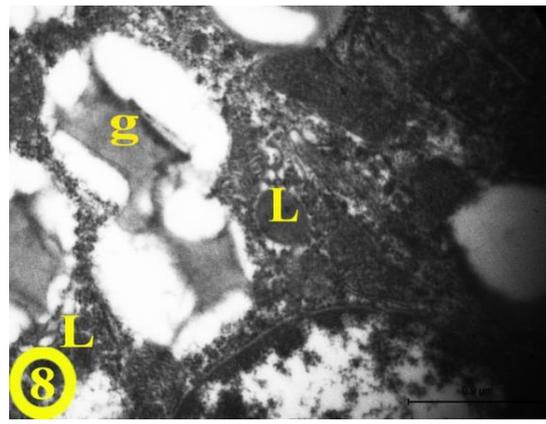
**Fig. (5)** Photomicrograph of a semithin section of the turkey epididymal region, showing proximal efferent ductules (PED) were lined with ciliated (C), non-ciliated cells type I (N) and basal cells with spherical ovoid nuclei (arrow). Toluidine blue X1000.



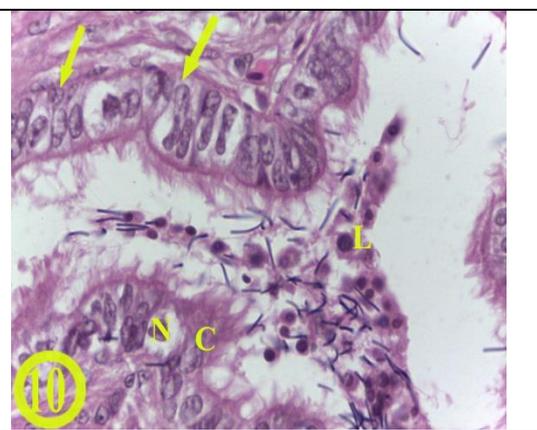
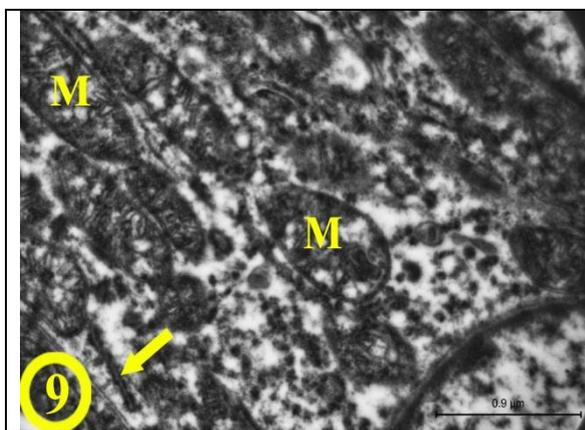
**Fig. (6)** Electron micrograph of the turkey epididymal region, showing proximal efferent ductules were lined with ciliated (C), non-ciliated cells type I (N) with supra and infranuclear cytoplasmic vacuoles containing granules and bleb like protrusions (arrow).Uranyl acetate and lead citrate X4000.

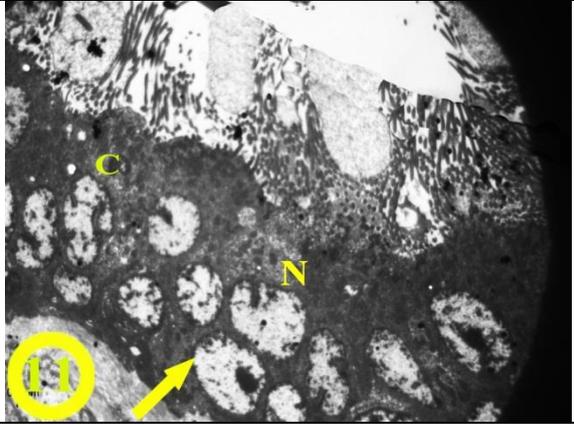
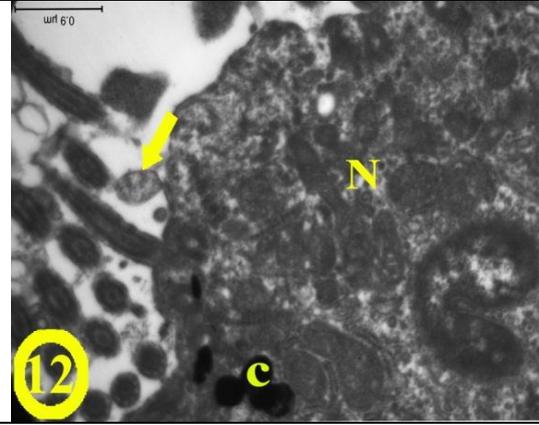


**Fig. (7)** Electron micrograph of the turkey epididymal region, showing junction complex (arrow) between ciliated (C) and non-ciliated cells type I (N). Uranyl acetate and lead citrate X30000.



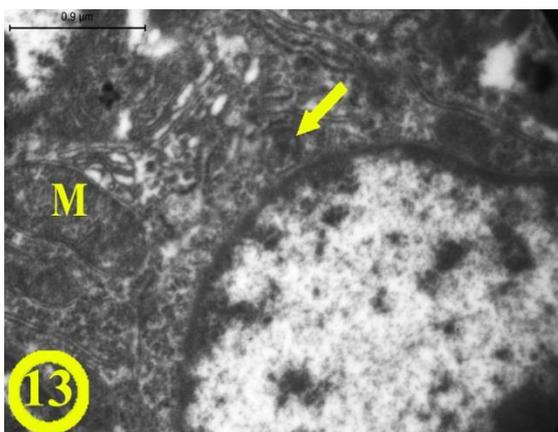
**Fig. (8)** Electron micrograph of the turkey epididymal region, showing vacuoles containing granules (g) and lipid droplets (L) in non-ciliated cells type I of the proximal efferent ductules. Uranyl acetate and lead citrate X30000.



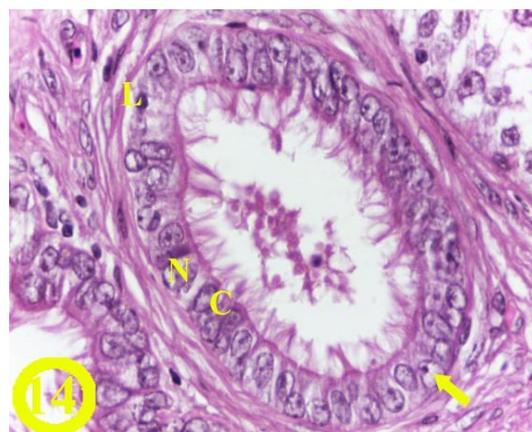
<p><b>Fig. (9) Electron micrograph of the turkey epididymal region, showing supra nuclear cytoplasm of the ciliated cells contained numerous mitochondria (M), rough endoplasmic reticulum (arrow) and free ribosomes Uranyl acetate and lead citrate X30000.</b></p>	<p><b>Fig. (10) Photomicrograph of a transverse section of the turkey epididymal region, showing the epithelium of distal efferent ductules was composed of non ciliated type II (N), numerous ciliated (C), basal cells (arrow). Notice intraluminal lymphocytes (L). H&amp;E X1000.</b></p>
	
<p><b>Fig. (11) Electron micrograph of the turkey epididymal region, showing non ciliated type II (N), numerous ciliated (C) and basal cells (arrow) Uranyl acetate and lead citrate X3000.</b></p>	<p><b>Fig. (12) Electron micrograph of the turkey epididymal region, showing non-ciliated cells type II (N), cytoplasmic blebs (arrow) and ciliated cells (C) Uranyl acetate and lead citrate X20000.</b></p>

**The distal efferent ductules:**

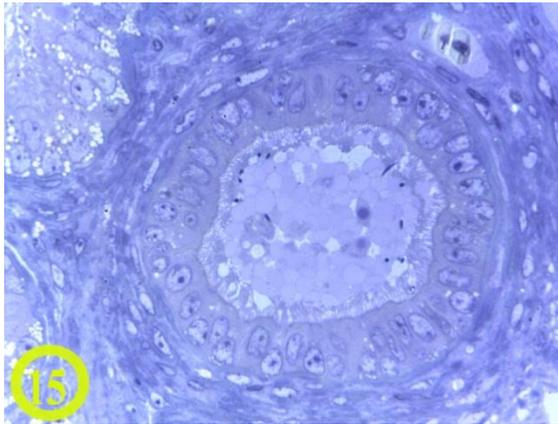
The distal efferent ductules were the continuation of the proximal efferent ductules. The epithelial lining was regular or less folded than the proximal efferent ductules (Fig. 1). The lumen contained a mass of spermatozoa. The epithelium was composed of non-ciliated type II, ciliated and basal cells. The ciliated cells were numerous than the non-ciliated one (Fig. 10). Additionally, intraluminal and interepithelial lymphocytes were found. Ultrastructurally the non-ciliated cells type II tend to form cytoplasmic blebs which projected into the lumen similar to type I of proximal efferent ductules (Figs. 11, 12). Ciliated cells possess similar characters of proximal efferent ductules (Fig. 13).



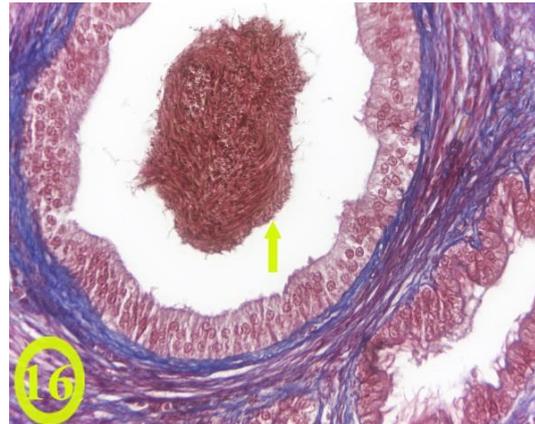
**Fig. (13) Electron micrograph of the turkey epididymal region, showing supra nuclear cytoplasm of ciliated cells possess mitochondria (M), free ribosomes and rough endoplasmic reticulum (arrow) Uranyl acetate and lead citrate X30000.**



**Fig. (14) Photomicrograph of a transverse section of the turkey epididymal region, showing narrow connecting duct were lined with ciliated (C), non-ciliated cells type III (N), basal cells (arrow). Notice interepithelial lymphocytes (L). H&E X1000.**



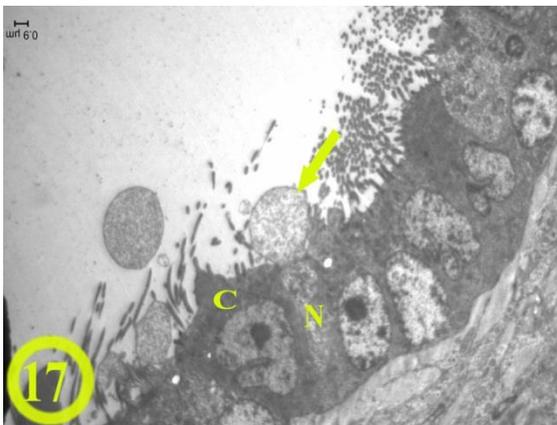
**Fig. (15)** Photomicrograph of a semithin section of the turkey epididymal region, showing toluidine blue positive granules in the lumina of narrow connecting duct. Toluidine blue X1000.



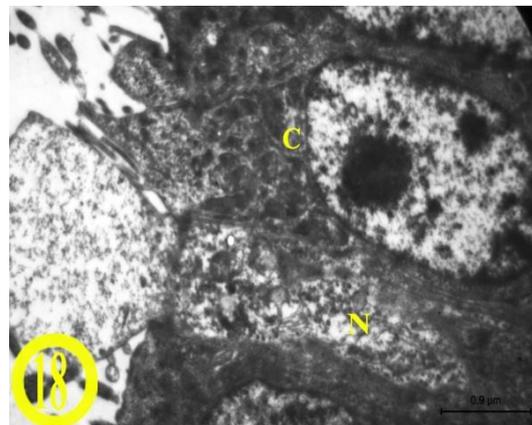
**Fig. (16)** Photomicrograph of a transverse section of the turkey epididymal region, showing mass of closely packed spermatozoa (arrow) in the lumen of the wide connecting duct. Masson's trichrome X400.

**The connecting ductules:**

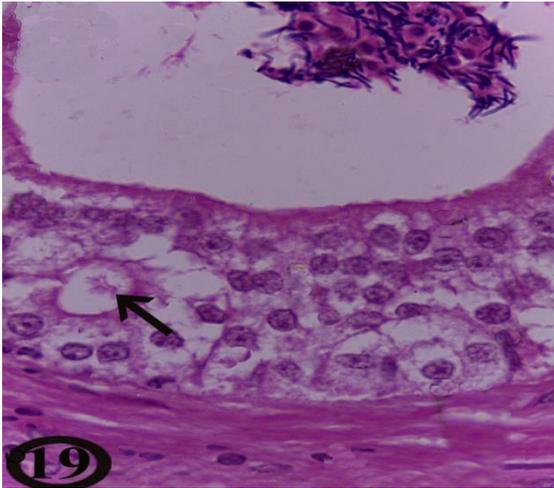
Narrow and wide connecting ductules were observed and lined with pseudostratified columnar cells. The height of the epithelium was regular and less than of the epididymal duct (Fig. 2). Ciliated cells were abundant in narrow connecting ductules. Intraluminal and interepithelial lymphocytes were presented (Fig. 14). The nuclei of the non-ciliated type III cells were basally, darkly stained and contained cytoplasmic granules which extruded into the lumina (Fig. 15). While ciliated cells were rarely seen in the wide connecting ductules and contained mass of closely packed spermatozoa (Fig. 16). Ultrastructural ciliated cells bearing a well formed tuft of cilia (Fig. 17). The principal epithelial cells of these duct was non ciliated type III which tend to form cytoplasmic blebs (Fig. 18).



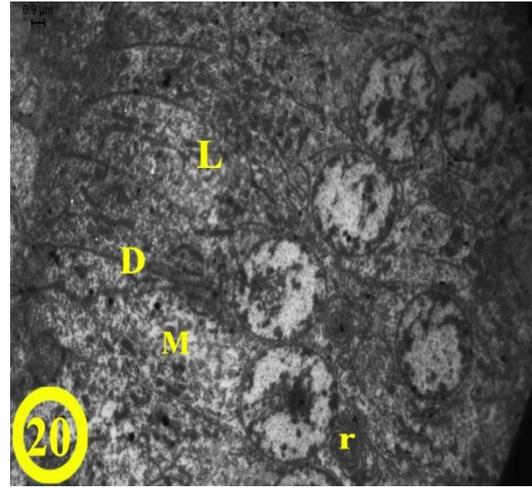
**Fig. (17)** Electron micrograph of the turkey epididymal region, showing ciliated (C), non-ciliated cells type III (N) with blebs like protrusions (arrow) of the connecting duct. Uranyl acetate and lead citrate X3000.



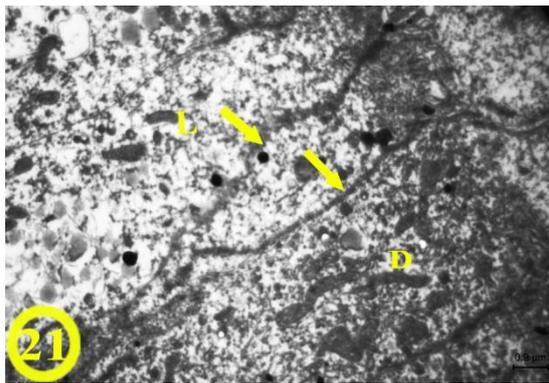
**Fig. (18)** Electron micrograph of the turkey epididymal region, showing ciliated (C), non-ciliated cells type III (N) which possess blebs like protrusions (arrow) of the connecting duct. Uranyl acetate and lead citrate X10000.



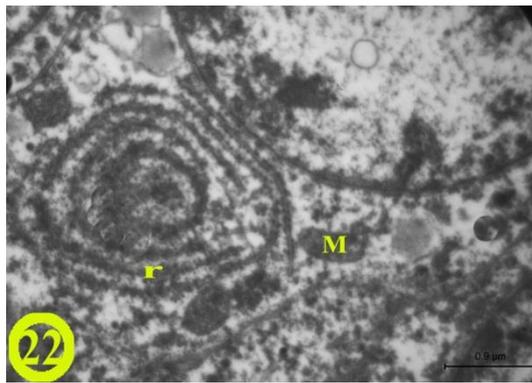
**Fig. (19)** Photomicrograph of a transverse section of the turkey epididymal region, showing epididymal duct lined by pseudostratified non ciliated columnar cells type III, basal cells. Notice intraepithelial glands containing eosinophilic material (arrow). H&E X1000.



**Fig. (20)** Electron micrograph of the turkey epididymal region, showing supra nuclear cytoplasm of the epididymal duct contained numerous mitochondria (M), rough endoplasmic reticulum (r), granules of dark cells (D) and light cells (L). Uranyl acetate and lead citrate X3000.



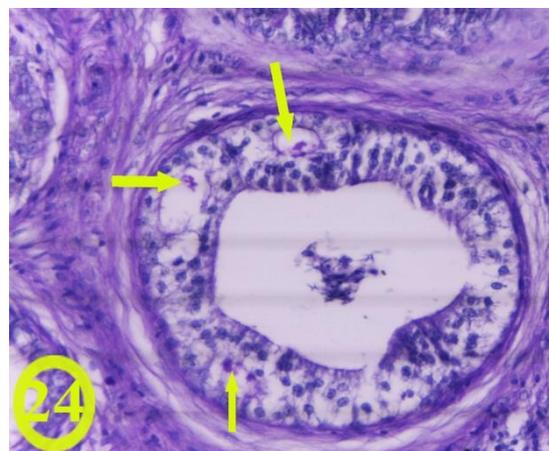
**Fig. (21)** Electron micrograph of the turkey epididymal region, showing junction complex (arrow) between dark cells (D) and light cells (L). Uranyl acetate and lead citrate X8000.



**Fig. (22)** Electron micrograph of the turkey epididymal region, showing infra nuclear rough endoplasmic reticulum (arrow) arranged as myelin like sheath. Uranyl acetate and lead citrate X20000.



**Fig. (23)** Photomicrograph of a transverse section



**Fig. (24)** Photomicrograph of a transverse

of the turkey epididymal region, showing supranuclear cytoplasm exhibit moderate PAS-positive reaction (arrow). PAS X1000.

section of the turkey epididymal region, showing intraepithelial glands of the epididymal ducts exhibit moderate PAS- positive reaction (arrow). PAS X400.

#### The epididymal duct:

It was lining similar to wide connecting ducts. More over intraepithelial glands containing eosinophilic material were also observed (Fig 19). Non ciliated cells exhibit same size, intensity, nuclear morphology with numerous cytoplasmic bleb-like extensions projected from the epithelium into the lumen. Supra nuclear cytoplasm contained numerous mitochondria, rough endoplasmic reticulum, granules of dark cells and light cells (Fig. 20). Junction complex appeared between these cells (Fig. 21). Infra nuclear rough endoplasmic reticulum arranged as myelin like sheath (Fig. 22).

#### Histochemical findings:

Neither reactivity for Alcian blue (pH 1 & 2.5), aldehyde fuchsin or Best's carmine was observed throughout the epididymal region of the turkey. While, a moderate PAS- positive reaction was demonstrated in the interductular connective, basement membranes of the rete channels and other ducts, the supranuclear cytoplasm and luminal borders of the proximal (Fig. 23), distal efferent ductular epithelium and intraepithelial glands of the epididymal ducts (Fig. 24).

### DISCUSSION

In the current study, the epididymal region of turkey could be demarcated into extra testicular rete, proximal efferent ductules, distal efferent ductules, connecting ductules, and the epididymal duct. This general structure was generally simulated that described for turkey, duck, Iranian white rooster and in pigeon (Hess et al., 1976; Sallam et al., 2001; Raziet al., 2010 and El-Saba and Abdrabou, 2013). On the other hand, Deshmukh et al. (2014) reported that the epididymis of a seel and vanaraja breeds of poultry was consisted of proximal and distal efferent ductules, connecting ductules and ductus epididymidis. Unlike the mammalian epididymis, it is not subdivided into head, body and tails reported in turkey (Hess et al., 1976), guinea fowl (Aire, et al., 1979) and dove Maruch, et al., 1996).

The squamous rete epithelium changed into high cuboidal or columnar type before the typical epithelium of the proximal efferent ductules began; similar results were reported by Aire (1982). On the contrary, Aire, et al. (1979) in guinea fowl observed that this change was abrupt from squamous epithelium to the high columnar cells of the efferent ductules. The cuboidal cells possibly are involved in secretion of proteinaceous fluids (Farquhar, 1969). The present study revealed that the connective tissue supported the rete channels contained lymphocytic aggregations; some of these aggregations took the nodular form. King and McLelland (1975) regarded it as normal in the domestic fowl and wild birds. Razi et al. (2010) suggested that these lymphocytes might add a more protective condition for the sperms. On the other hand, lymphocytic aggregation had developed a number of different immunological strategies including cell mediated one in fowl Sharnl-k (1997).

Few smooth muscle fibres were seen in the wall of epididymis as reported in Iranian white rooster (Razi et al., 2010) and seel and vanaraja breeds of poultry (Deshmukh et al., 2014). These contractile cells in interductal tissue in the ostrich appear to be necessary for onward propulsion of spermatozoa (Aire et al., 2008).

The efferent ductules consisted of two portions: the proximal and distal efferent ductules which came in agreement with (Sallam et al., 2001; Razi et al., 2010; El-Saba and Abdrabou, 2013 and Deshmukh et al., 2014). On the other hand, Tingari (1971) did not observe such division in the fowl but he classified them as efferent ductules and narrow connecting ducts, respectively. The ultrastructure of turkey's epididymis followed (Aire et al., 1979; Sallam et al., 2001 and El-Saba and Abdrabou, 2013) in their classification of the non-ciliated cells of the proximal efferent ductules as type I and that of the distal efferent ductules as non-ciliated cells type II.

The current results agree with Razi et al.(2010), which suggest that the highly developed efferent ductules play a particularly critical and important role in fluid reabsorption and removal of particulate or protein material from the lumen whereas the distal segment may be involved in only fluid reabsorption. This speculation is in consonance with the organelle content in the supranuclear region of the non-ciliated (type I) cell of the proximal efferent duct, where numerous and well-formed tubular coated pits, apical tubules, dilated vacuoles, and dense bodies abound. In the distal segment, on the other hand, these organelles are don't elaborated in the type II cells as in the type I cells of the proximal segment. The highly folded mucosa of the proximal efferent duct indicated a large ratio of luminal surface area to luminal volume which was obviously consistent with fluid reabsorption activity (Clulow and Jones, 1988). Aire (1980) added that the activity of type I cell in resorption is more than type II cell. Such resorption may offer an explanation for the great concentration of spermatozoa in the distal efferent ductules and connecting ductules. El- rafey(1985) suggested that the testicular fluid could be an unsuitable vehicle for sperm maturation in the epididymis and must be resorped for concentration. The type I cells in the proximal efferent ductules of the turkey contained abundant mitochondria with a concentric arrangement around lipid vesicles and rough endoplasmic reticulum. Such associations indicate that these cells manufacture proteins, possibly albumen, which is found in turkey seminal plasma (Thurston, 1976). Lin and Chang (1975) had shown that albumen appears to be synthesized by bound polysomes and released directly into the cytoplasm. The release of protein into the lumen could be accomplished by apocrine secretion, which may account for the cytoplasmic blebs. These blebs represented a sign of apocrine secretion (Sallam et al., 2001; El- Saba and Abdrabou, 2013). The intra luminal released secretory cells had been observed in turkey's efferent ductules were suggested to be mature holocrine secretory cells (Martanand Risley, 1963 and Martanand Allen, 1964) in mouse epididymis. The last authors assumed that the presence of holocrine cells in the epididymis establishes a secretory function of the organ these functions are likely related to the sperm maturation and maintenance in the epididymis. Morphological evidence of the steroid secretory activities was indicated in the efferent ductules of the turkey by the presence of vacuoles, long cisternae of rough endoplasmic reticulum and numerous lipid vesicles as in duck (Sallam et al., 2001) and pigeon (El- Saba and Abdrabou, 2013).

The present findings support the speculation in different birds (Tingari and Lake, 1972; Aire, 1979 and Aire, 1980) that the morphological features of the ciliated cells indicated their positive participation in phagocytosis and digestion of broken down germ cells and degenerated spermatozoa as well as, pinocytosis of most of the fluid entering the epididymal region from the testes. In rat, an extensive phagocytosis of spermatozoa cleared the lumen and permits the continual movement of the sperms along the tract. These study noticed PAS positive granules in the supra-nuclear cytoplasm of efferent ductules as well as rER and secretory vesicles (Cooper and Hamilton, 1977). These secretory products might be needed for sperm nutrition (Sallam et al., 2001; El- Saba and Abdrabou, 2013). The PAS reaction was moderate as observed in drakes (Lee and Ha, 1983). While mild PAS activity was seen in a seel (Deshmukh et al., 2014). In the current study macrophages observed in the lumen of the rete channels and in the intraepithelial lining of the efferent ductules in agreement with Yeung et al. (1994) who recorded that spermiphagy occurred by luminal and tissue macrophages are among the factors concerned in the disposal of an ejaculated or degenerated spermatozoa in the epididymal region. In this respect, Aruldas et al. (2010) postulated that the basal cells of the human epididymis might transform into macrophages. The connecting ductules were at first narrow, but as they approached the epididymal duct began to anastomose with each other and thus became progressively wider. They end by joining the single epididymal duct as recorded here (Lake, 1981).

The connecting duct and ductus epididymidis epithelium lined with pseudo stratified columnar and basal cells. The main cell in both ducts was the non-ciliated (Type III) cell. This investigation revealed that narrow connecting ductules consist mainly of ciliated cells while wide consist of non-ciliated of light and dark cells and basal similar to the epididymal duct (Tingari, 1972). Whenever, that connecting duct is one compartment and possessed the same structure of the epididymal duct, although it was larger in diameter and lined with non-ciliated cells (Lake, 1981; Rikihisa and Lin, 1988; Sallam et al., 2001 and El- Saba and Abdrabou, 2013). Meanwhile, connecting ductules were also lined by pseudostratified epithelium with less cilia in fowl (Tingari, 1971), in Iranian white rooster (Razi et al., 2010) and a seel as compared to vanaraja (Deshmukh et al., 2014). Ductuli efferentes and connecting ductules presumably serve to transport spermatozoa (Deshmukh et al., 2014 and Aire and Josling, 2000). In the current study, the intraepithelial gland was observed in epididymal duct and gave PAS positive reaction. Abd El-maksoud (2010) and Ahmed (2013) in camel observed that the intraepithelial gland was formed when the number of vacuolated basal cells aggregated in certain region within the epithelium. The vacuoles of the basal cells were close to each other and finally coalesced to

form a single large cavity. This cavity contained secretory homogenous substance that gave strong PAS positive reaction.

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