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HISTOLOGICAL, HISTOCHEMICAL, AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE EFFERENT DUCTULES OF THE DOVE

Fatma El-Zahraa Ahmed Mustafa[1, *], and Basim. S. A. Al Sulivany[2]

[1]Department of Cell and Tissues, Faculty of Veterinary. Medicine, Assiut University, Assiut, 71526, Egypt.

[2]Biology Department, College of Science, University of Zakho, Zakho, 42002, Duhok, Kurdistan Region, Iraq.

[*]Corresponding author email: f.histology@aun.edu.eg

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ABSTRACT:

This research examines the structural features of the efferent ductules (EDs) in healthy doves (*Spilopelia senegalensis*) collected from local hunters in Assiut, Egypt. After slaughter, the efferent ductules were promptly dissected and preserved in Bouin's fluid. Utilizing a combination of histological, histochemical, and immunohistochemical techniques, we explored the architecture of EDs, focusing on the stroma and parenchyma. The lining epithelium was made up of a simple columnar to the pseudostratified columnar epithelium, ciliated, secretory, and basal cells. However, this study is the first to identify telocytes within the peritubular region of the dove's efferent ductules, highlighting their potential role in tissue communication and regeneration. Additionally, androgen receptor expression was detected in the epithelial cells and spermatozoa within the lumen, suggesting a significant role in the male reproductive system.

KEYWORDS: Apocrine Secretion; Telocyte; Sperms; Lymphocyte; Androgen.

1. INTRODUCTION

The Laughing Dove (LD; *Spilopelia senegalensis*) is a small bird belonging to the Columbidae family and distributed in Africa. (Madkour & Mohamed, 2019). Only a limited number of prior studies have documented the breeding patterns of birds in Africa. The LD exhibits reproductive activity throughout the year without being influenced by seasonal changes (Earlé & Dean, 1981). The ductuli efferentes, or efferent ductules, are tiny tubules that connect the rete testis and the epididymis. These ducts possess distinctiveness because they are the sole portion within the male reproductive tract that contains different important structural components (Aire, 2002).

Efferent ductules (Eds), considered a simple conduit for the spermatozoa from the testis to the epididymis, have recently gained greater significance for their role in sperm processing, such as the absorption of fluid through processes such as active solute transport, passive permeability, fluid-phase endocytosis, and adsorptive endocytosis, leading to an increase in the concentration of spermatozoa (Ilio & Hess, 1994).

Little information is available about the structure of efferent ductula in birds, and there is no available literature about the structure of efferent ductula in doves, which pushed us to study this part and try to fill the scientific gap. This work studies different structural components, including the stroma and different types of connective tissue fibers present in EDs. In addition, parenchyma and different cellular components are described in detail, including the lining epithelium; ciliated cells, secretory cells, basal cells, immune cells, intraepithelial lymphocytes, and interstitial lymphocytes, and telocytes, which, as newly described interstitial cells, play an important function in cellular communication. Moreover, they maintain heaemeostaisi and help in tissue regeneration. Also, this work used different histological methods to support the data, including general histological staining, histochemical staining, and immunohistochemical techniques.

2. MATERIALS AND METHODS

Sample Collection:

The samples were obtained by local hunters in the Assiut governorate, Egypt. The specimens were collected from five healthy laughing doves (*Spilopelia senegalensis*). The study received approval from the Ethics Committee of Assiut University, Egypt, under Code number 06/2024/0250. Following the slaughtering process, the efferent ductules were rapidly dissected and preserved in Bouin's solution (Gorgees *et al.*, 2013).

Histological Examination:

After being dehydrated in ethyl alcohol and cleared with methyl benzoate, the fixed specimens were embedded in paraffin wax. According to Bancroft *et al.* (2013), samples were cut at a thickness of 3-6µm and stained using the following methods: Harris haematoxylin and eosin, Crossmon's trichrome, Grimelus silver, and Wigert's Elastica.

Immunohistochemistry:

The immunohistochemical identification of the Androgen Receptor in paraffin sections was conducted following the protocol described by Hsu *et al.* (1981), utilizing the Androgen Receptor Monoclonal Antibody (AR 441) (Catalog # MA5-

* Corresponding author

13426). The efferent ductule sections (4um) were subjected to xylene dewaxing, followed by ethanol rehydration. Subsequently, the sections were rinsed in phosphate buffer saline (PBS) with a pH of 7.4, repeating this process three times for 5 minutes each time. The activity of endogenous peroxidase was suppressed by adding 1% hydrogen peroxide at room temperature for 10 minutes. The sections were rinsed in PBS (pH 7.4) for 5 minutes. They were then placed in a sodium citrate buffer with a concentration of 10 mM and a pH of 6.0. After that, they were heated at a temperature range of 95-98°C for 20 minutes in a water bath. After heating, they were allowed to cool down to room temperature. The slides were washed in PBS solution with a pH of 7.4. Then, they were exposed to the primary antibodies for 30 minutes at room temperature. The sections were rinsed with PBS (pH 7.4) for 5 minutes. Next, they were exposed to the secondary antibody for 10 minutes at room temperature. The slides were washed in PBS (pH 7.4) and then treated with drops of streptavidin-peroxidase complex (Thermo Fisher Scientific, USA) for 10 minutes at room temperature. The slices were washed in PBS (pH 7.4). They were stained with Harris hematoxylin as a counterstain. Subsequently, they were dried, clarified, and mounted using DPX.

3. RESULTS

The efferent ductules were enveloped by a delicate connective tissue capsule, primarily composed of collagen fibers. Furthermore, many elastic fibers were discovered on the capsule, oriented in different directions. The interstitial tissue was composed primarily of collagen fibers with a few elastic fibers. Smooth muscle fibers (SMF) are found in bundles at a subcapsular position. These bundles of muscles are covered by collagen and elastic fibers (Fig. 1A-B-C-D-E-F). The interstitial tissue mainly consists of collagen, elastic, and reticular fibers, together with various types of cells, blood vessels, and lymph vessels (Fig. 2A-B-C-D-E).

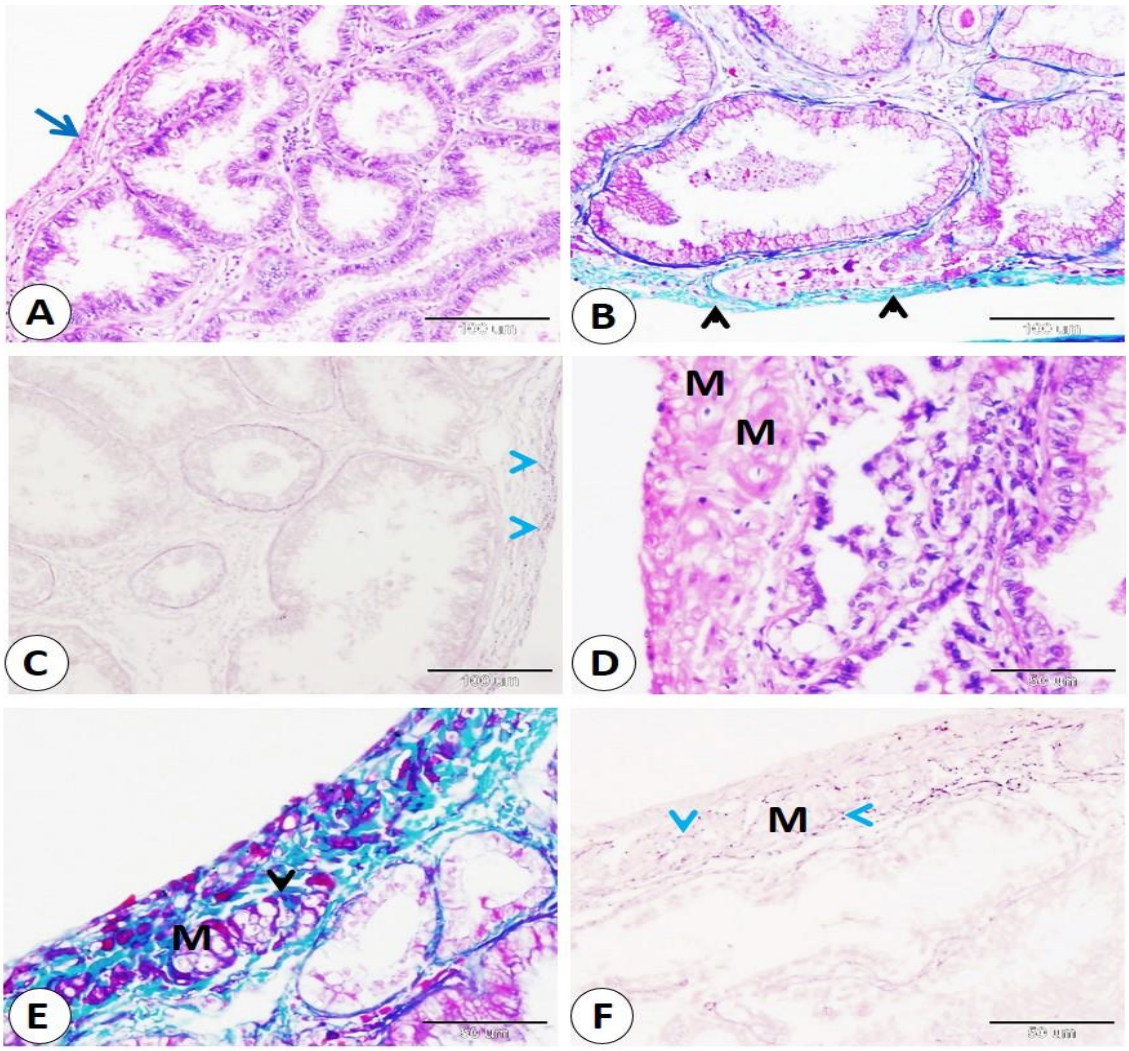

Figure 1: Stroma of the efferent ductile general structure. A: Capsule (blue arrow) (HE). B: Collagenous fibers (black arrowheads) (Crossmon's trichrome). C: capsule with elastic fibers in different directions (blue arrowheads) (Weigert's resorcin fuchsin). D: SMFs in subcapsular position (M) (HE). E: SMFs (M) surrounded by collagenous fibers (black arrowhead) (Crossmon's trichrome). F: SMFs (M) surrounded by elastic fibers (blue arrowheads) (Weigert's resorcin fuchsin).

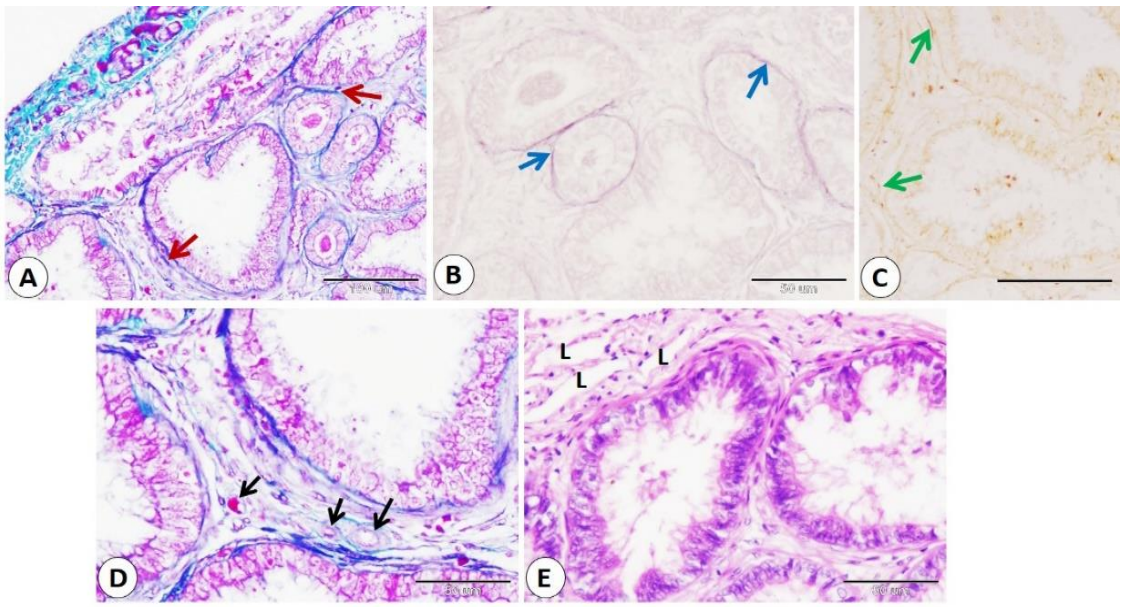

Figure 2: The general structure of the efferent ductile Stroma. A: collagen fibers at interstitial tissue (red arrows) (Crossmon's trichrome). B: elastic fibers at interstitial tissue (blue arrows) (Weigert's resorcin fuchsin). C: reticular fibers at interstitial tissue (green arrows) (Grimelus silver). D: interstitial blood vessels (black arrows) (Crossmon's trichrome). E: lymph vessels (L) (HE).

The efferent ductule's parenchyma consists primarily of tubules of varying sizes. The interstitial space between the tubules contains various types of interstitial cells. The epithelium lining the efferent ductules exhibited a columnar to a pseudostratified ciliated columnar epithelial structure. The columnar cells were categorized into ciliated and secretory cells. The ciliated cells are the most prominent cell type characterised by lightly stained, acidophilic, large columnar cells with long cilia. They had rounded to oval nuclei and were situated centrally or basally within the cell. The dark cells were characterized by their thin columnar shape, deeply stained acidophilic cytoplasm, and elongated nucleoli. These cells exhibited apocrine secretion, and the blebs were detected in the lumen of the ductules. Basal cells are few and appear as small, rounded pyramidal cells found on the same basement membrane as columnar cells with rounded nuclei. Intraepithelial lymphocytes were detected among the previously reported cells located at various positions. Furthermore, larger ducts show epithelial overcrowding. The lumen of the efferent ductules in doves contains spermatozoa and blebs, which are produced through apocrine secretion by the secretory cells lining the efferent ductules. The efferent ductules were surrounded by one to three layers of smooth muscle fibers (Fig. 3A-B-C-D-E).

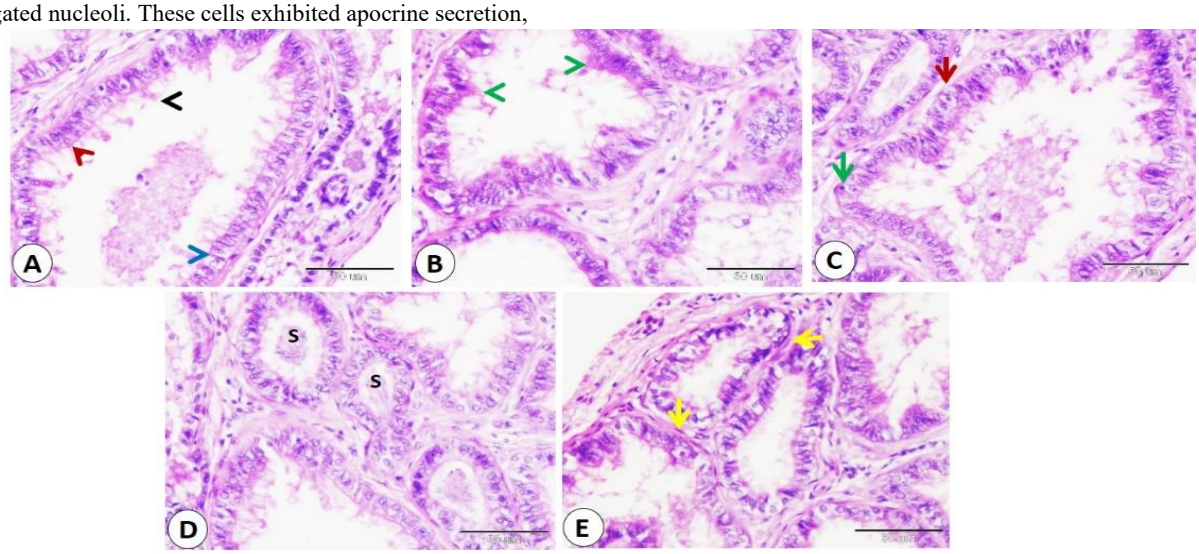

Figure3: The parenchyma of the efferent ductule (A-B-C-D). Ciliated (red arrowhead), secretory cells (blue arrowhead), Ciliated cells (red arrowhead), blebs (black arrowhead), epithelial overcrowdings (green arrowheads), basal cell (red arrow), intraepithelial lymphocyte (green arrow), sperms (S), and smooth muscle fibers (yellow arrows). (HE).

A positive reaction of PAS-AB staining was noted on the epithelium lining the efferent ducts, as well as on the blebs adhering to the apical border of the cell and within the blebs in the lumen. A positive reaction to the same stain was also found at the efferent ductules' basement membrane. In addition, the presence of an argyrophilic reaction was observed either individually or in groups of cells in the lining epithelium of the efferent ductules, as well as in the interstitial cells. Furthermore, argyrophilic granules are evenly dispersed on the epithelium lining the efferent ducts. The efferent ductile lumen exhibited an argyrophilic response in the sperms (Fig. 4A-B-C-D). Various cellular components, such as fibroblasts, lymphocytes, and telocytes, have been identified in the interstitial tissue of the dove efferent duct. Telocytes are positioned in the peritubular region and have elongated cell bodies and expanded telopods (Figure 5A-B-C-D). In addition, the presence of Androgen receptor immunohistochemical reactivity was detected in the efferent ductile. Also observed was a slight to strong reactivity to androgen in the lining epithelium, strong reactivity to androgen in overcrowded areas with cells, and strong reactivity to androgen in the sperms within the lumen (Fig. 6A-B-C).

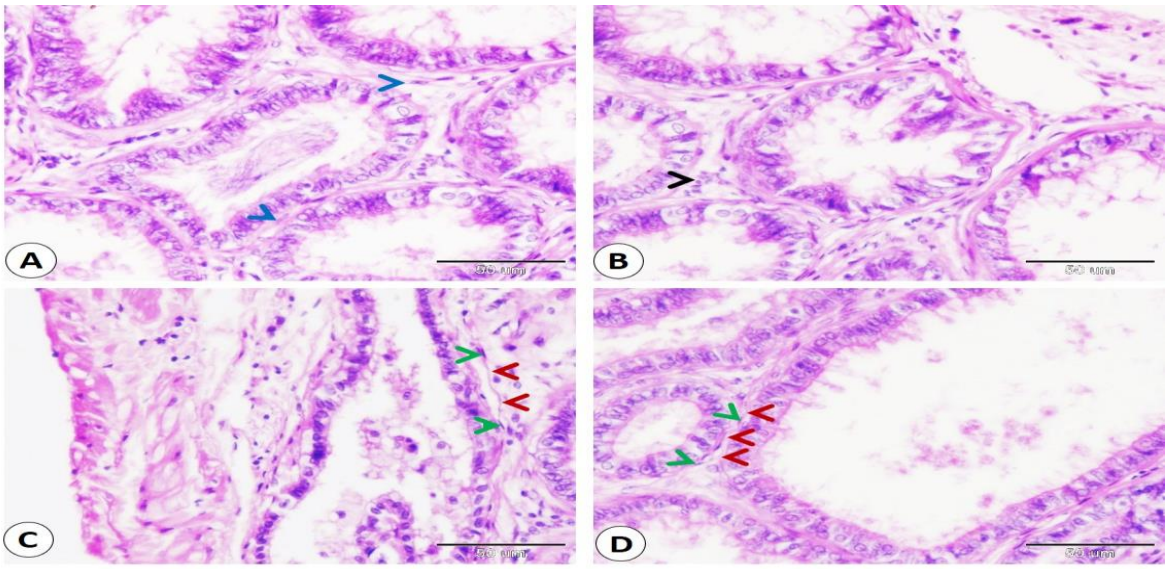

Figure 4: Efferent ductile with PAS-AB satin (A-B) and Grimelus silver (C-D). A-B: PAS-AB satin positive reaction on the blebs (green arrowheads) and in the basement membrane (green arrow). C-D: Argyrophilic reaction in the lining epithelium (blue arrows), in the interstitial cells (green arrow), argyrophilic granules distributed on the lining epithelium (black arrow), and Argyrophilic reaction on the sperm in the lumen (S).

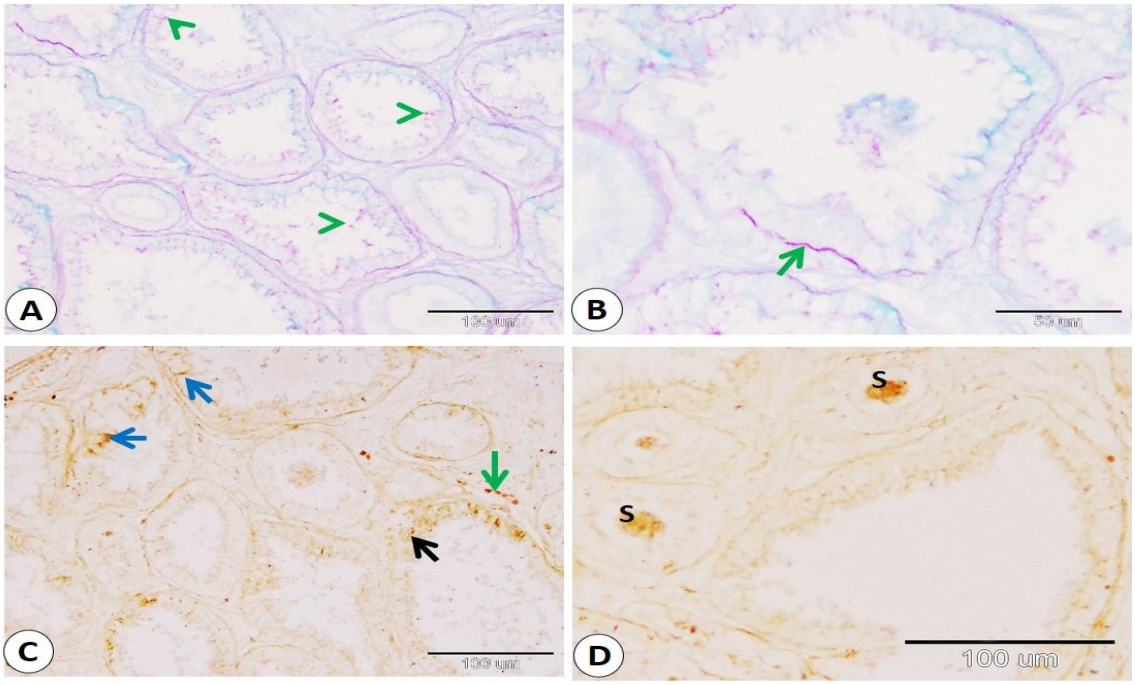

Figure 5: interstitial cells in efferent ductile with HE stains. A: fibroblast (blue arrowheads). B: lymphocyte (black arrowhead). C-D: cell body of telocyte (green arrowheads) and telopods (red arrowheads).

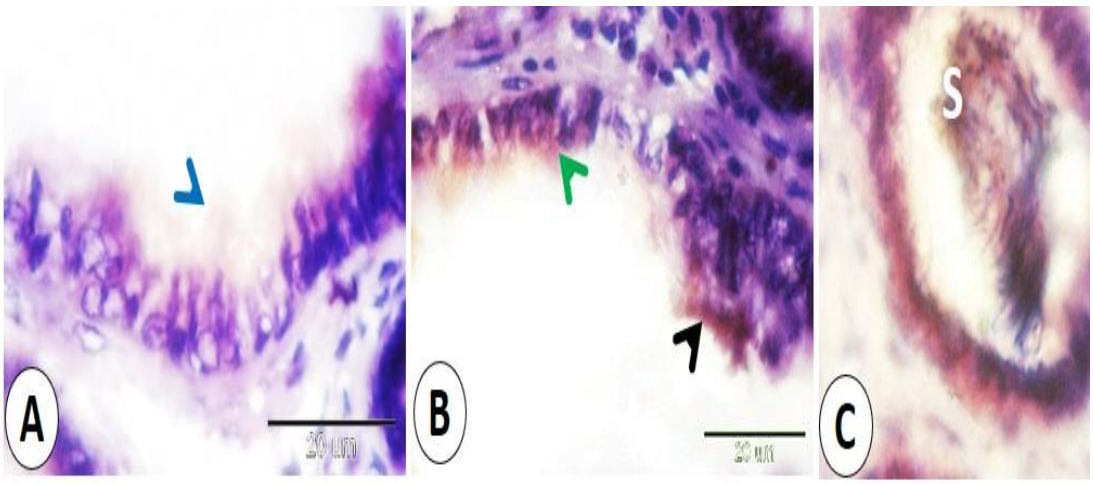

Figure 6: Androgen receptors in the lining epithelium of efferent ductule (A-B-C). Slight (blue arrowhead) to strong (green arrowhead) reactivity to androgen at the lining epithelium, strong reactivity to androgen at overcrowded areas with cells (black arrowhead), and strong reactivity to androgen on the sperms at the lumen (S).

4. DISCUSSION

The present work focused on the structure of the efferent ductile on the dove, which is composed mainly of the stroma and parenchyma. The stroma of the efferent ductile is formed of a connective tissue capsule with subcapsular smooth muscle fibers and interstitial tissue, rich in collagen and elastic fibers with few reticular fibers. Moreover, no distinct septa were detected in the efferent ductile of the dove. The parenchyma consists of tubules of different sizes and interstitial cells.

The present study revealed that the lining epithelium of efferent ductiles was columnar to pseudostratified columnar. This result agrees with those described in different birds' efferent ductiles: Quail (Ibrahim *et al*., 2022), Pigeon (Stefanini *et al.*, 1999), and guinea fowl (Aire *et al*., 1979). In addition, the present work found different types of cells that lined the efferent ductules of doves: ciliated, secretory, and basal cells. Similar observations were detected by Abd-Elmaksoud *et al*. (2009) in the study of ducks and turkeys by Hess *et al.* (1976). In the present results, ciliated cells were the most prominent cell type. This agrees with different species of ciliated cells, as in duck (Abd-Elmaksoud *et al.*, 2009), and pigeon (Stefanini *et al*., 1999). Non-ciliated cells were responsible architecturally for the reabsorption of more than 80% of the fluid that enters the epididymis from the testis in the Japanese quail and the ostrich efferent ductules (Aire, 1980; Aire & Soley, 2000). The present study also found apical blebs at the apical border of the secretory cells, a similar observation described in different birds, and considered these criteria an indication of the apocrine mode of secretion of these cells in turkey and chicken (Bakst, 1980). The present work indicated that basal cells were few basally located cells; this agrees with the findings of Tingari. (1971) in domestic fowl and Stefanini *et al.* (1999) in pigeons. Also, the present data indicate the sperms in the lumen of efferent ductules showed an argyrophilic reaction. Previously isolated sperms from the testes and semen of cattle and pigs showed argyrophilic reactions (Andraszek & Smalec, 2011).

The lymphocyte described in this paper is in the intraepithelial position and is one of the cells present in the interstitium. The lymphocyte is described in the epididymis as one of the important immune cells that play a key role in maintaining male immunity and is sometimes referred to as hallo cells (Zhao *et al.,* 2020). Also, lymphocytes in the excurrent duct of the testes may have a significant effect on suppressing an autoimmune response to spermatozoa by acting as an immunological barrier (Yakirevich *et al.*, 2002).

In the available literature, no telocytes have been detected in the efferent ductile of birds. So, the present work first detected efferent ductule telocytes in doves in this research in the peritubular region. In our previous paper, telocytes were detected on the testes of doves (Mustafa & Elhanbaly, 2021). Also, telocyte observed previously on different organs of different animals (Mustafa & El-Desoky,2020; Mustafa, & Elhanbaly, 2020; Soliman, Abd-Elhafeez, Abou-Elhamd, Kamel, Abdellah, & Mustafa; 2023). Telocytes are mesenchymal origin cells with a small cell body and varying numbers of lengthy prolongations termed telopodes. A cell body's shape might be spindle-shaped, triangular, or stellate, depending on how many telopodes it has (Pawlicki *et al.*, 2024). Telocytes exhibit the capacity to engage with many neighbouring cells to form both homo- and hetero-cellular connections, which contribute to the structural support and organization of tissues throughout development and homeostasis (Cretoiu & Popescu, 2014)

The development of the male reproductive system involves many factors, androgen being one of these important. Androgen performs its function by binding to the androgen receptor (Welsh *et al.*, 2012). Previous studies have demonstrated the expression of AR in the testes of doves, but no studies have recorded its efferent ductile (Mustafa & Elhanbaly, 2021). The present work detected AR expression in cells lining the efferent ductile and in the spermatozoa within its lumen. However, AR expression was observed in different animal species in the prostate, epididymis, and testis (Abate-Shen & Shen, 2000; Zhu *et al.*, 2000).

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Competing Interests:
The authors declare that they have no competing interests.

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