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Original Article

Histological, Histochemical, and Ultrastructural Approach to the Ductus Deferens in Male Nile Monitor Lizard (*Varanus niloticus*)

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Abstract

The ductus deferens is a fundamental part of the male genital tract and the continuation of the epididymal duct. As a male secondary sex organ, the ductus deferens plays a crucial role in the nourishment, storage, and maturation of spermatozoa. Some studies have provided information about the ductus deferens structure in reptiles; however, the full description of the ductus deferens remains to be clarified. The current study aimed to describe the Nile monitor lizard (*Varanus niloticus)* ductus deferens from histological, histochemical, and ultrastructural perspectives. The results revealed that the ductus deferens is formed histologically from two main cell types: principal and basal. The principal cells were tall and filled with periodic acid Schiff (+)/alcian blue (−) cytoplasmic granules. The basal cells were found just above the basement membrane. By transmission electron microscopy, the principal cells exhibited typical protein-secreting cell features. Additionally, some intraepithelial cells, such as halo cells, undifferentiated mesenchymal cells, and agranular leukocytes, were identified. This study presents the first detailed description of the *Varanus niloticus* ductus deferens. Further immunohistochemical studies are required to explore the function(s) of the cellular components.

Key words: agranular leukocyte, ductus deferens, halo cell, mesenchymal cell, monitor lizard, reptiles

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Introduction

One of the largest and most widely distributed lizard species in Africa is the Nile monitor lizard (*Varanus niloticus)*. These lizards are active freshwater carnivorous predators (Luiselli et al., 1999; Thompson, 1999) that live for up to 15 years (de Buffrenil et al., 1994). Egyptian Nile monitor lizards live in different habitats close to the Nile River that vary from grasslands to forests. Research focusing on the Egyptian Nile monitor lizard was initiated in 2013 by Moustafa et al. (2013) who studied the lizard's hematological and biochemical parameters. Being a carnivorous predator reptile, Nile monitor lizards play an important role in the ecosystem and control of some other animal species (King & Green, 1999; Bennett, 2002). In Egypt, the Nile monitor lizards live close to both human and domestic animals (Moustafa et al., 2013). Understanding the structure and function of the Nile monitor lizard's genital systems may enable control of its reproduction. Generally, the male reptile's genital system consists of the testes (the site of spermatogenesis and spermiogenesis) and the deferens ducts that are composed of efferent ductules, the epididymis, and ductus deferens. The ductus deferens extends from the epididymis to the common cloaca at the base of the penis (Wyneken & Mader, 2002; Guerrero et al., 2004). A description of the male Nile monitor lizard ductus deferens structure would be useful in the assessment of their reproductive performance. To enhance the understanding of the Nile monitor lizard reproductive physiology, the aim of this study was to fully describe the Nile monitor lizard ductus deferens from histological, histochemical, and ultrastructural perspectives.

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Materials and Methods

Collection of Ductus Deferens

Three apparently healthy adult male Nile monitor lizards were captured alive in the vicinity of Qena city, Qena province, Egypt during summer (August 2018). The rostro-anal length and weight were 130 ± 22 cm and 9 ± 2 kg, respectively. All animals were euthanized as soon as possible after catching, as previously described (Awad & Mohamadain, 2019). The ductus deferens that ran along the ventral surface of the kidney parallel with the ureter were collected and washed with normal saline solution (NaCl 0.9%). Within 1 h from the time of collection, the samples were transported to the laboratory in 10% formalin for histological study. The procedures of the current study were approved by the Animal Care and Use Standards Ethics Committee, South Valley University.

Histological Preparation

Upon arrival in the laboratory, the samples were fixed in 10% neutral-buffered formalin. After fixation, the specimens were dehydrated in ascending grades of ethanol, cleared in xylene,

and finally embedded in Paraplast (Sigma-Aldrich, St. Louis, MO, USA). A microtome was used to cut tissue sections of 5–7 μm in thickness (Reichert Leica RM 2125 Microtome, Germany) (Bancroft & Layton, 2013).

Histological and Histochemical Staining Protocols

Sections were deparaffinized using xylene (two times for 1 h each), then rehydrated in descending grades of ethanol (100, 90, 80, and 70%) and distilled water. Hematoxylin and eosin (H&E) (Harris, 1900) were applied for ordinary histological examinations. The sections staining reactivity was tested with special stains using periodic acid Schiff (PAS)/alcian blue (AB) combination stain (Mowry, 1956) for the detection of acidic and neutral mucins, Safranin O stain (Tran et al., 2000) for the detection of mucin, and Crossmon's trichrome technique for the differentiation of connective tissue and muscle fibers (Crossmon, 1937). After staining, the sections were dehydrated again in an ascending series of ethanol (70, 80, 90, and 100%), cleared in xylene twice for 1 h each, and mounted with distyrene dibutyl phthalate (DPX).

Semi-Thin Sectioning

The proximal portion of the ductus was fixed in 2.5% glutaraldehyde in sodium (Na) phosphate buffer saline at 4 °C overnight, followed by washing four times (15 min/each) in 0.1 M sodium phosphate buffer (pH 7.2). Subsequently, they were postfixed in 1% osmic acid in 0.1 M sodium phosphate buffer at 4 °C for 2 h. Rewashing was performed using 0.1 M phosphate buffer (pH 7.2) (three times, 20 min each). The fixed specimens were dehydrated using serial concentrations of alcohols: 70% (1 h minimum), 90% (30 min), and two changes of 100% (15 min each). The samples were permeated with a mixture of normal Spurr's resin and 100% acetone overnight (1:1), then during the day (2:1), and finally embedded in pure Spurr's resin and allowed to polymerize (48 h at 60 °C). Semi-thin sections (0.5 μm) were cut with an ultramicrotome (LEICA ULTRACUT UCT), stained with 1% toluidine blue in 1% borax (Burns, 1978), and viewed with a light microscope.

Transmission Electron Microscopy

Ultrathin sections (70 nm) were obtained using a Reichert ultramicrotome. Using uranyl acetate and lead citrate, sections were stained and examined by JEOL100CX II transmission electron microscope at the Electron Microscopy Unit, South Valley University, Qena, Egypt (Burns, 1978).

Results

Anatomical, Histological, and Histochemical Features of the Nile Monitor Lizard Ductus Deferens

Grossly, the ductal portion of the Nile monitor lizard ductus deferens was located along the ventral surface of the kidney parallel to the ureter (Fig. 1a). The ductus deferens appeared to be oriented into several cross-sections of various shapes and sizes. It was separated from the ureteral and renal tissues by connective tissue (Figs. 1b, 1c). The lumen of the ductus deferens was slightly folded and filled with sperm (Figs. 2a–2d). The ducts were lined with nonciliated simple columnar cells filled with PAS-positive/AB-negative secretory granules (Fig. 2f). The epithelium was completely surrounded by several layers of smooth muscle fibers with indistinct connective tissue adventitia in between (Figs. 2e–2h).

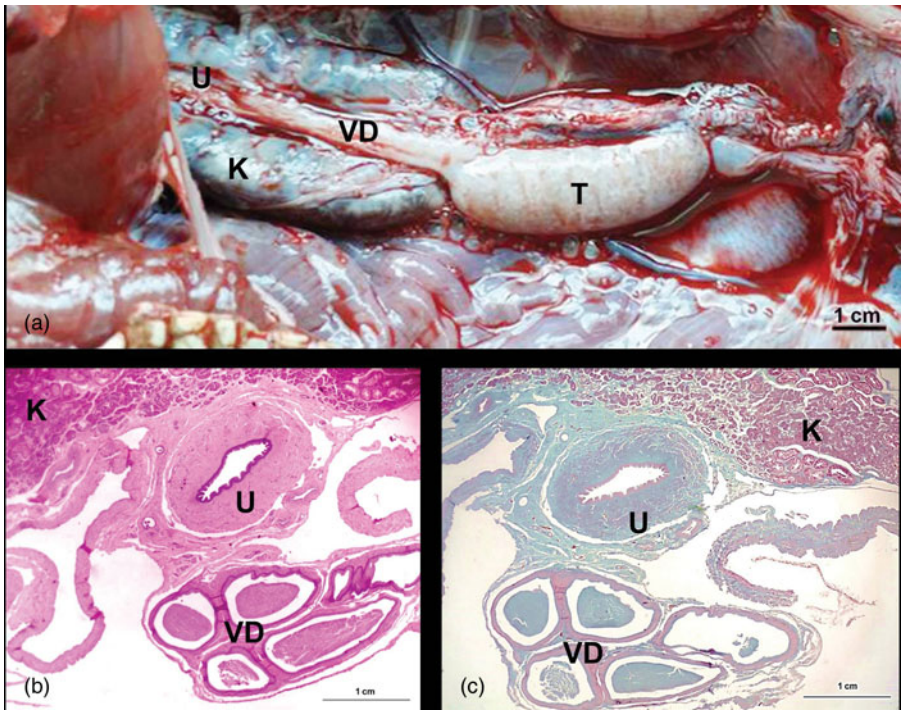

Fig. 1. The topographical features of ductal portion in Nile monitor lizard vas deferens. (**a**) Showing the anatomical position of the ductus deferens. (**b,c**) Displaying the histological location of the ductus deferens stained by H&E and Crossmon's trichrome, respectively. K, kidney; T, testis; U, ureter; VD, vas deferens. Scale bars = 1 cm.

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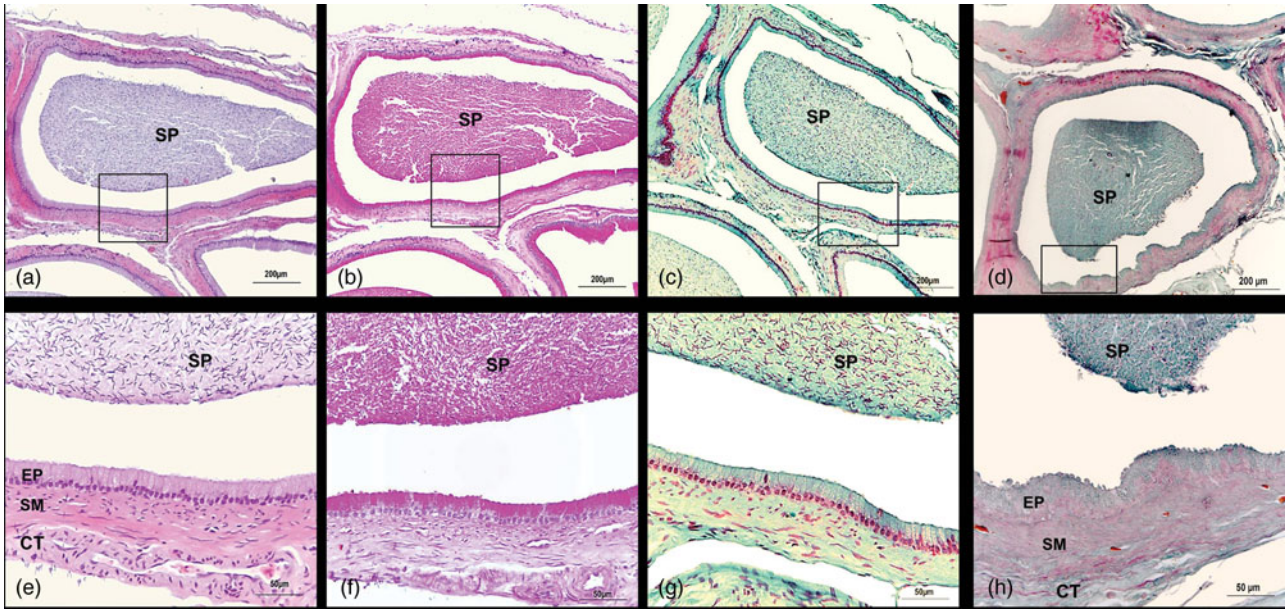

Fig. 2. The histological structure of ductal portion in Nile monitor lizard vas deferens stained with H&E (**a**), PAS–AB (**b**), Safranin O (**c**), and Crossmon's trichrome (**d**). (**e–h**) represent the higher magnification of the frame in (**a–d**), respectively. The lumen of the ductus deferens filled with sperms (SP). CT, connective tissue; EP, epithelium; SM, smooth muscle fibers. Scale bars = 200 μm in (**a–d**) and 50 μm in (**e–h**).

Semi-Thin Characteristic Features of the Nile Monitor Lizard Ductus Deferens

The semi-thin sections revealed that the ductus deferens was histologically formed from two main cell types: principal and basal (Fig. 3). The principal cells were tall and showed different stages of secretion, varying from granule free to moderate and fully occupied cells with cytoplasmic secretory granules (Fig. 3). The basal cells were small with a light and vesicular nucleus and located just above the basement membrane. In addition, a few apical cells with large oval nuclei were identified in some semi-thin sections (Fig. 3a). The ductus deferens epithelium exhibited various levels of apocrine exocytotic secretion of groups or individual cytoplasmic granules toward the lumen that was PAS-positive/AB-negative (Fig. 4).

Ultrastructure of the Nile Monitor Lizard Ductus Deferens

The ultrastructure of the two main cell types, principal cells and basal cells, of the Nile monitor lizard ductus deferens was clarified. The principal tall cells reached the epithelial surface and were packed with a huge number of electron-dense secretory granules (Fig. 5a). Moreover, the principal cells had active vesicular euchromatic nuclei and exhibited typical protein-secreting cell features with well-developed cytoplasmic organelles, such as rough endoplasmic reticulum, Golgi apparatus, numerous rod-shaped mitochondria, secretory vesicles of various sizes, and lysosomes (Figs. 5c–5e). Furthermore, the ultrastructure of the ductus deferens revealed other scarce intraepithelial cells, such as halo cells, undifferentiated mesenchymal cell, and agranular leukocyte. The halo cell was located basally with a flattened nucleus

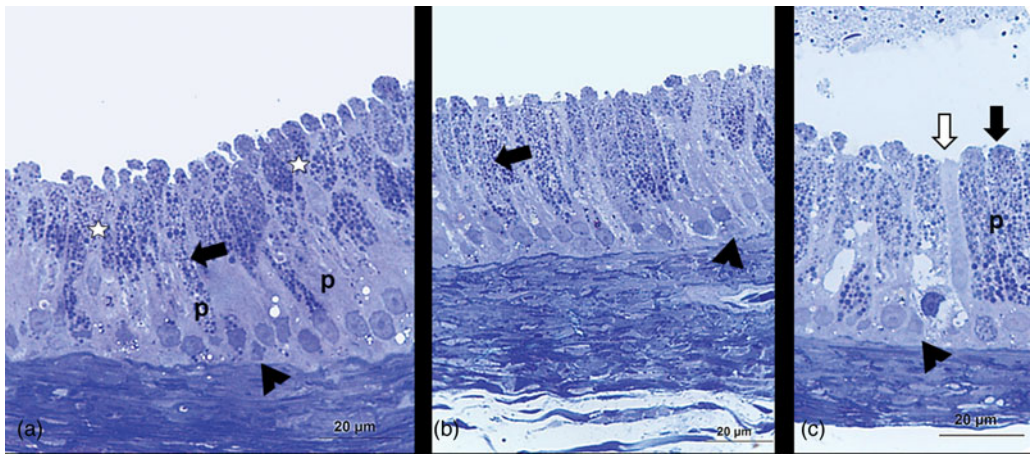

Fig. 3. Semi-thin sections of ductal portion in Nile monitor lizard vas deferens. The ductus deferens formed from two main basic types of cells: principle tall cell (p) and basal cell (arrowhead). The principal cells showed various stages of cytoplasmic secretory granules varied from granular free (white arrow), moderate and fully occupied cells (back arrow). Few apical cells with large oval nucleus and cytoplasmic granules (star). Scale bars = 20 μm.

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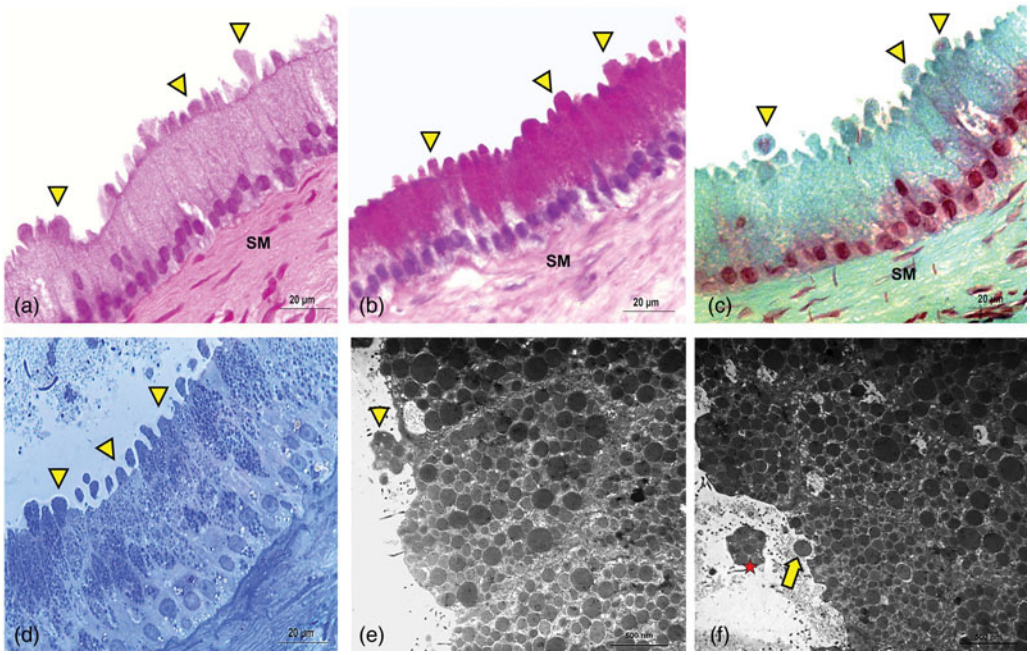

Fig. 4. Secretory mechanism of ductal portion in Nile monitor lizard vas deferens. (**a–d**) showed the apocrine secretory mechanism (arrowheads) either histological sections stained with H&E, PAS–AB, and Safranin O or semi-thin with toluidine blue, respectively. (**e,f**) Electromicrographs displayed the secretory vesicles either in groups (star) or individual vesicle (arrow). SM, smooth muscle fibers. Scale bars = 20 μm in (**a–d**) and 500 nm in (**e,f**).

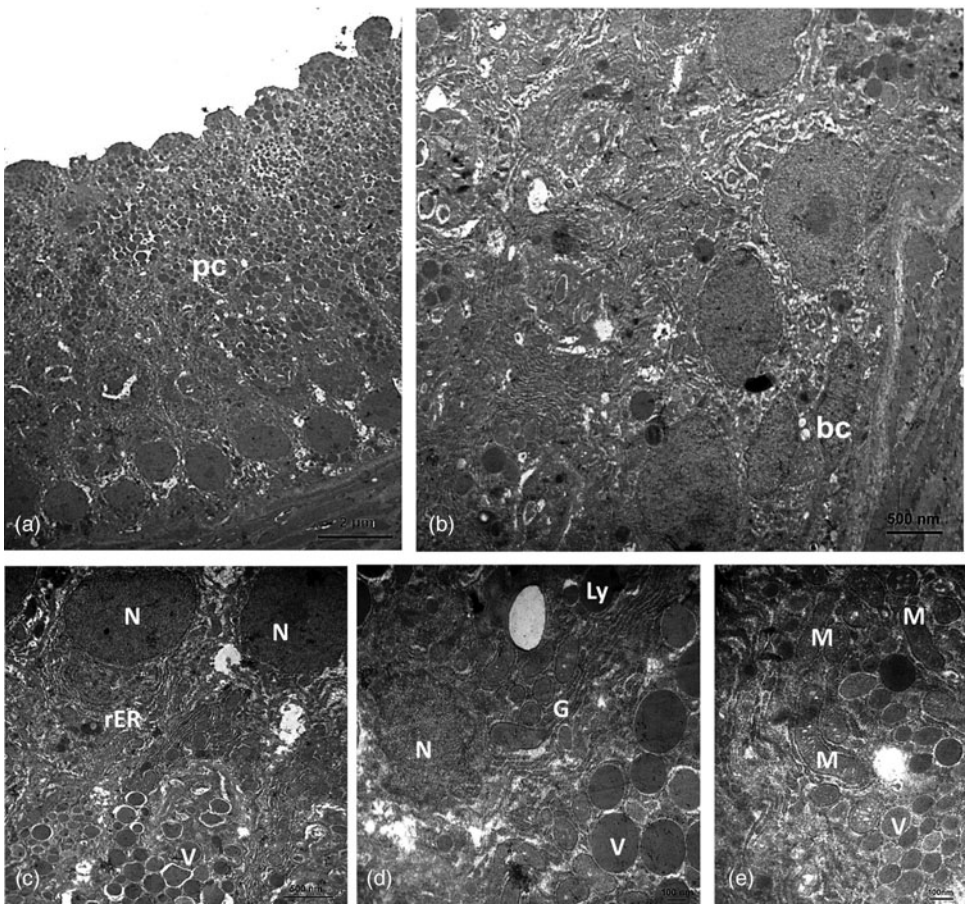

Fig. 5. Electromicrographs of epithelium lining ductal portion in Nile monitor lizard vas deferens. Two main basic types of cells: principle tall cell (pc) and basal cell (bc). Principle cells showed typical protein secreting cell components filled with intracytoplasmic secretory vesicle (V). G, Golgi apparatus; Ly, lysosome; M, mitochondria; N, nucleus; rER, rough endoplasmic reticulum. Scale bars = 2 μm in (**a**), 500 nm in (**b,c**), and 100 nm in (**d,e**).

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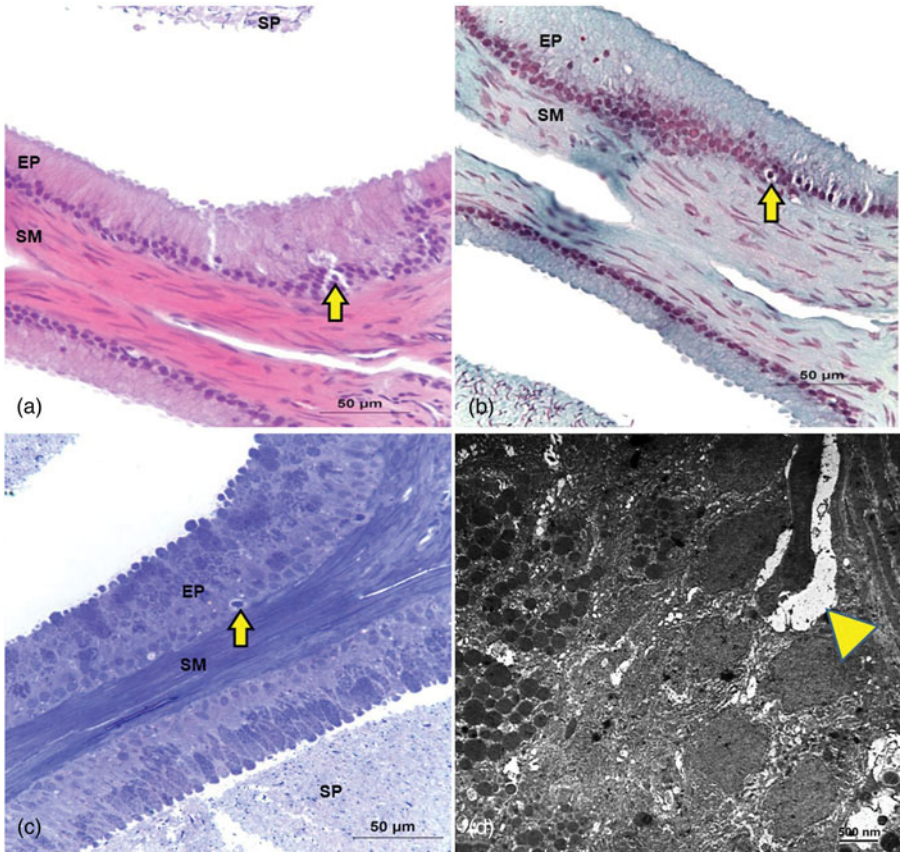

Fig. 6. Localization of halo cell in the epithelium lining ductal portion of Nile monitor lizard vas deferens. (**a–c**) showed halo cell (arrow) located near the basement membrane either in histological sections stained with H&E and Safranin O or semi-thin with toluidine blue, respectively. (**d**) Electromicrograph displayed the halo cell with flatted nucleus surrounded by a halo space (arrowhead). EP, epithelium; SM, smooth muscle fibers; SP, sperms. Scale bars = 50 μm in (**a–c**) and 500 nm in (**d**).

surrounded by a halo space (Fig. 6). The undifferentiated mesenchymal cell was characterized by a large, divided nucleus with high nuclear to cytoplasmic ratio, a basal interdigitation with the basement membrane, rough endoplasmic reticulum (rER), and dispersed ribosomes (Fig. 7a). Agranular leukocyte was located just above the basement membrane and was characterized by large u-shaped nuclei (Figs. 7b, 7c).

Discussion

The anatomical location of the ductus deferens in relation to the ventral surface of the kidney in the male Nile monitor lizard was similar to that previously reported in other reptiles (Guerrero et al., 2004; Viana et al., 2014). In this study, two main types of cells, principal tall cells and basal cells, could be histologically identified in the ductus deferens of male Nile monitor lizards. This finding shows similarity to that previously reported in water monitor lizards (*Varanus salvator bivittatus*) (Mahfud et al., 2016). In this study, the detection of a large number of spermatozoa filling the lumen of the ductus deferens strongly supports the idea that the ductus deferens acts as the main sperm storage site in male Nile monitor lizards. Relatively, long-term storage of spermatozoa in the ductus deferens likely increases the reproduction rate by extending the breeding time.

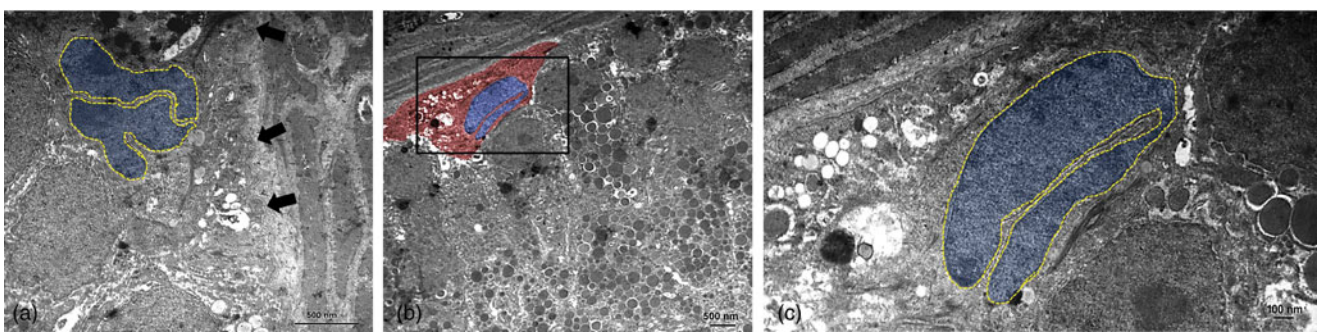

Fig. 7. Electromicrographs of intraepithelial cells in ductal portion of Nile monitor lizard vas deferens. (**a**) Intraepithelial undifferentiated mesenchymal cell with dividing nucleus and basal interdigitation with the basement membrane (arrows). (**b**) Intraepithelial agranular leukocyte resting on the basement membrane with characteristic u-shape like nucleus. (**c**) represents the higher magnification of the frame in (**b**). Scale bars = 500 nm in (**a,b**) and 100 nm in (**c**).

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Interestingly, histochemical characterization of the secretory granules in the ductus deferens epithelium revealed PAS-positive/AB-negative granules. These secretions would be a glycogen because of their neutral mucin nature. Recently, a PAS-positive/AB-negative neutral mucoid secretion was also detected in the epithelium lining the sexual segment of the male Egyptian Nile monitor lizard kidney. This sexual segment of the lizard kidney is suggested to have a role in vital reproductive processes such as spermatogenesis and testosterone production (Awad & Mohamadain, 2019).

In contrast to Viana et al. (2014) and Mahfud et al. (2016), who reported that the epithelium lining other reptile ductus deferens is ciliated, no cilia could be detected in the epithelium lining the ductus deferens in the male Egyptian Nile monitor lizard in the present study. Nonciliated epithelium supports the hypothesis that the main function is storage, not transportation. The transportation function required to transfer the spermatozoa to the cloaca is maintained by the contraction of the several layers of smooth muscle that, as reported in the present study, completely surround the ductus deferens epithelium.

Apocrine exocytotic secretions of PAS-positive/AB-negative cytoplasmic granules, detected by semi-thin sectioning, possibly play a nutritive role for the stored spermatozoa in the ductus deferens lumen. The reason that principal tall cells secrete protein, and the roles of the other intraepithelial cells reported here, namely halo cells, undifferentiated mesenchymal cell, and agranular leukocytes, needs to be clarified in future studies.

Conclusion

In conclusion, the ductus deferens in male Egyptian Nile monitor lizards consists of two main cell types (principal tall cells and basal cells) and other scarce intraepithelial cells (halo cells, undifferentiated mesenchymal cell, and agranular leukocytes). The ductus deferens is lined with nonciliated columnar secretory epithelium cells with PAS-positive/AB-negative characteristics. This ductus deferens in male Nile monitor lizards performs several critical roles in addition to the transport of spermatozoa from the epididymis to the cloaca; it stores spermatozoa and supports spermatozoa nourishment and maturation.

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Conflict of interest. The authors declare that they have no conflicts of interest

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