Proximal Testicular Ducts of the Mediterranean Gecko (Hemidactylus turcicus)

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ABSTRACT

The efferent ducts of the Mediterranean Gecko, Hemidactylus turcicus (Gekkonidae) were investigated using light and electron microscopy. The seminiferous tubules unite into a single rete testis tubule. The rete testis divides into 3–4 ductuli efferentes which all drain into the cranial portion of the ductus epididymis. All efferent ducts are most active during the months of December to August. The rete testis is composed of a simple squamous epithelium with bifurcated nuclei and a labyrinthine network of intercellular canaliculi. Ciliated and nonciliated cells are present, and more than one cilium extends from the scattered ciliated cells. The presence of small clear vesicles and widened intercellular canaliculi suggest that cells of the rete testis are responsible for intake of luminal fluids. The ductuli efferentes are composed of a simple cuboidal epithelium consisting of ciliated and nonciliated cells, and ciliated cells are the dominant cell type. During the inactive season the number of lysosomes increases and the cells become spermiophagic. The ductus epididymis is composed of a tall pseudostratified columnar epithelium with relatively scarce basal cells. No evidence for regionalization was observed. The ductus epididymis is highly secretory during the active season with numerous electron-dense secretory granules, whose glycoprotein products are released by merocrine secretion. Basally, the active epididymis has swollen intercellular canaliculi and enlarged cisternae of rough endoplasmic reticulum. During the inactive season the secretory activity decreases and membranous structures and fibrous material are observed within widened intercellular canaliculi apical to the basal cells. Anat Rec, 293:2176–2192, 2010. © 2010 Wiley-Liss, Inc.

Key words: Rete testis; epididymis; testis; ultrastructure; Gecko

INTRODUCTION

From the seminiferous tubules of squamate reptiles (i.e., lizards and snakes), sperm sequentially pass through the rete testis, ductuli efferentes, ductus efferens, and ampulla ductus deferentis (Akbarsha et al., 2006a, 2007; Sever 2010). Martin-Saint-Ange (Fig. 1) was the first to note that the rete testis in a lizard, Lacerta vivipara, consists of a single extratesticular tubule that unites with the ductuli efferentes: "En ce point, on voit se détacher du testicle deux ou trois petits tubes, rarement plus, qui, après un tronc très-court, se réunissent en un seul conduit. C’est là que commence l’épididyme" (Martin-Saint-Ange, 1854: p. 67). Note that Martin-Saint-Ange (1854) did not use the modern terms “rete testis” or “ductuli efferentes,” but his striking illustration, reprinted here as Figure 1,
makes our interpretation quite clear. Martin-Saint-Ange’s description also indicates that two or more intra-testicular rete tubules branch from the seminiferous tubules. Subsequently, Alverdes (1926) reported from four species of *Lacerta* that a single rete tubule “der einzige Hodenausfu¨hrungsgang” passes into a longitudinal canal “langskanal” where 10–18 ductuli efferentes “vasa efferentia” connect to the ductus epididymis, particularly at the cranial end.

Both Van den Broek (1933) and Fox (1977) illustrated the same nonhistological drawing of the proximal testicular ducts of *Lacerta* that a single rete tubule “der einzige Hodenausfu¨hrungsgang” passes into a longitudinal canal “langskanal” where 10–18 ductuli efferentes “vasa efferentia” connect to the ductus epididymis, particularly at the cranial end.

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Volsøe (1944) proposed that the ancestral condition for the squamate rete testis occurs in snakes, in which the rete testis has many tubules along the entire length of the testis, and no longitudinal duct occurs. Lizards display a more derived condition and “only one or a few ducts remain” (Volsøe, 1944, p. 77). Volsøe (1944) argued that *Anguis* represents a “transitional” stage. The observations on the rete testis of lizards cited in Table 1 have been limited to light microscopy, and only one study (Sever, 2010) described the ultrastructure of the rete testis of a snake.

The ductuli efferentes of lizards have also been largely ignored. Aside from the 10-18 tubules of the ductuli efferentes mentioned for *Lacerta* by Alverdes (1926), Reynolds (1943) reported 11 in the skink *Eumeces fasciatus*, Averal et al. (1992) observed 7–8 in the agamid *Calotes versicolor*, and Akbarsha et al. (2007) found 6–8 in another agamid, *Sitana ponticeriana*. Some workers, such as Forbes (1941) apparently considered the ductuli efferentes to comprise the initial segment of the ductus epididymis. The only ultrastructural study on the ductuli efferentes in a lizard is by Akbarsha et al. (2007) on *S. ponticeriana*.

The ductus epididymis of squamates has received more attention than other proximal testicular ducts. The most comprehensive review of the histology and histochemistry is by Dufaure and Saint Girons (1984) who

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**TABLE 1. Number of tubules in the rete testis and presence of a longitudinal canal in Squamates**

<table>
<thead>
<tr>
<th>Family</th>
<th>Rete testis</th>
<th>Longitudinal</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agamidae</td>
<td>Sitana ponticeriana</td>
<td>One</td>
<td>No</td>
</tr>
<tr>
<td>Anguidae</td>
<td>Anguis fragilis</td>
<td>Five-nine</td>
<td>Yes</td>
</tr>
<tr>
<td>Iguanidae</td>
<td>Sceloporous undulatus</td>
<td>One</td>
<td>No</td>
</tr>
<tr>
<td>Lacertidae</td>
<td>Lacerta sp.</td>
<td>One</td>
<td>Yes</td>
</tr>
<tr>
<td>Lizards</td>
<td>Eumeces fasciatus</td>
<td>One</td>
<td>Yes</td>
</tr>
<tr>
<td>Scincidae</td>
<td>Chalcides ocellatus</td>
<td>Nine</td>
<td>No</td>
</tr>
<tr>
<td>Scincus scincus</td>
<td>Six</td>
<td>Yes</td>
<td>Badir, 1958</td>
</tr>
<tr>
<td>Serpentes</td>
<td>Natrix natrix</td>
<td>Three-33</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Seminatrix pygaea</td>
<td>Five-seven</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Thamnophis sp.</td>
<td>Numerous</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Vipera sp.</td>
<td>Numerous</td>
<td>No</td>
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</table>
examined the ductus epididymides of 89 species of squamates, representing 72 genera and 18 families. These authors reported five types of secretory activity which ranged from none (characterizing snakes) to copious large secretory granules (Lacertidae). Ultrastructure studies exist on species from three families of lizards, and these vary in the number of zones or cell types recognized, even among species within the same family (Table 2).

Data on the proximal testicular ducts of lizards of the family Gekkonidae, a cosmopolitan family of over 1000 species, are scarce. Henry (1900) lists the Mediterranean Gecko, Hemidactylus flaviviridis, as one of the reptiles he examined in his study on secretory activity of the epididymis in higher vertebrates, but his descriptions largely assessed Lacerta agilis, with additional observations included for L. muralis, L. vivipara, and Anguis fragilis. He only mentions two types of anterior testicular ducts, “les petits tubes,” which match the description of the ductuli efferentes, and “les gros tubes,” which is the ductus epididymis. Henry (1900) found no evidence of secretory activity in the ductuli efferentes, but found seasonal variation in secretory activity in the ductus epididymis, with numerous secretory granules in April-June. Dufaure and Saint Girons (1984) included some geckos in their comparative study of secretory activity in the epididymis. They found either small secretory granules or finely granulated cytoplasm in species from nine genera (Gekko, Geckonia, Stenodactylus, Tarentola, Gehrya, Heteronota, Hoplodactylus, Oedure, and Coleonyx).

Haider and Rai (1981) reported on the epididymis in Hemidactylus flaviviridis, although no ultrastructural data were provided. These authors reported three regions in the epididymis (anterior, middle, and posterior) although it appears that these three regions correspond to the ductuli efferentes, ductus epididymis, and ductus deferens respectively. In this article, we present our findings on the histology and ultrastructure of the proximal testicular ducts of Hemidactylus flaviviridis introduced to Louisiana. Our results are the first ultrastructural descriptions of the rete testis, ductuli efferentes, and ductus epididymis of squamates.

**MATERIALS AND METHODS**

**Animal Collection**

Adult male Mediterranean Geckos, Hemidactylus turcicus, were collected from Hammond, Louisiana monthly from March 2006 to March 2008. Animals were measured (snout-vent length and total length), euthanized via an intraperitoneal injection of sodium pentobarbital (method approved by the Institutional Animal Care and Use Committee at Southeastern Louisiana University), and dissected within 2 days of capture. Reproductive tracts (testis, kidneys, and efferent ducts) were removed and the right side was placed in 10% neutral buffered formalin for light microscopy and the left side was placed in Trump’s fixative (2.5% glutaraldehyde and 2.5% formaldehyde in

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### Table 2. Ultrastructural studies on the ductus epididymides of Squamata

<table>
<thead>
<tr>
<th>Family</th>
<th>Zones</th>
<th>Cell types</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agamidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calotes versicolor</td>
<td>Proximal caput</td>
<td>Principal</td>
<td>Averal et al. (1992)</td>
</tr>
<tr>
<td></td>
<td>Distal caput</td>
<td>Narrow</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proximal corpus</td>
<td>Apical</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distal corpus</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cauda</td>
<td>Dark</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Basal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Initial segment</td>
<td>Principal</td>
<td>Meeran et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Caput</td>
<td>Narrow</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Corpus</td>
<td>Apical</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cauda</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Basal</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Halo</td>
<td></td>
</tr>
<tr>
<td>Sitana ponticerana</td>
<td>Initial segment</td>
<td>Principal</td>
<td>Akbarsha et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Caput</td>
<td>Narrow</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Corpus</td>
<td>Apical</td>
<td></td>
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<tr>
<td></td>
<td>Cauda</td>
<td>Clear</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Basal</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intraepithelial leukocytes</td>
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<tr>
<td>Lacertidae</td>
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<td>Lacerta vivipara</td>
<td>Caput</td>
<td>Principal</td>
<td>Mesure et al. (1991)</td>
</tr>
<tr>
<td></td>
<td>Proximal corpus</td>
<td>Basal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distal corpus</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cauda</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Podarcis sicula</td>
<td>Caput</td>
<td>Principal</td>
<td>Desantis et al. (2002)</td>
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<tr>
<td></td>
<td>Corpus</td>
<td>Basal</td>
<td></td>
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<tr>
<td></td>
<td>Cauda</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tropiduridae</td>
<td>No zones</td>
<td>Secretory</td>
<td>Ferreira et al. (2009)</td>
</tr>
<tr>
<td>Tropidurus itambere</td>
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<td>Basal</td>
<td></td>
</tr>
<tr>
<td>Serpentes</td>
<td>No zones</td>
<td>Principal</td>
<td>Sever (2010)</td>
</tr>
<tr>
<td>Seminatrix pygaea</td>
<td></td>
<td>Basal</td>
<td></td>
</tr>
</tbody>
</table>
0.1 M sodium cacodylate buffer at pH 7.4; Electron Microscopy Sciences, Hatfield, PA) for electron microscopy. Carcasses are catalogued into the Vertebrate Museum of Southeastern Louisiana University.

**Light Microscopy**

Tissues were fixed in formalin for 48 hrs before being rinsed with de-ionized water, dehydrated through a graded series of ethanol solutions (70, 80, 95, 100%), cleared in two changes of toluene, and placed in melted paraffin for 24 hrs under vacuum. Tissues were then embedded in paraffin blocks and allowed to harden overnight. Serial sections were cut at 10 μm on a RMC microtome (RMC Instruments, Tucson, AZ), placed on albuminized slides, and alternate slides were stained with hematoxylin and eosin (general cytology), bromphenol blue (proteins), or periodic acid and Schiff’s reagent (PAS, neutral carbohydrates) counterstained with alcian blue 8GX at pH 2.5 (carboxylated glycosaminoglycans) (Hayat, 1993). Slides were viewed using a Leica DM2000 compound microscope, photographs were taken with a Leica DF420 attached digital camera (Leica Microsystems, Wetzlar, Germany), and composite micrographs were constructed using Adobe Photoshop 7.0 (Adobe Systems, San Francisco, CA).

**Electron Microscopy**

Tissues fixed in Trump’s fixative and stored at 4°C were rinsed with de-ionized water, post-fixed in 2% osmium tetoxide (diluted from 4% using deionized water) at room temperature before being treated with 0.1 M sodium cacodylate buffer at pH 7.4; Electron Microscopy Sciences, Hatfield, PA) for electron microscopy.

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Fig. 2. Light microscopy of the reproductive system stained with hematoxylin and eosin. (A) Overview of the entire reproductive system showing the seminiferous tubules (St), rete testis (Rt), adrenal gland (Ag), ductuli efferentes (De), and epididymis (Ep). Scale = 400 μm. (B) Enlarged view of the seminiferous tubules (St) and the single extratesticular rete testis (Rt). Scale = 50 μm. (C) Enlarged view of the ductuli efferentes (De) showing the ciliated squamous epithelium. Scale = 50 μm. (D) Enlarged view of the epididymis with sperm (Sp) present in the lumen. Scale = 50 μm.
temperature, and dehydrated through a graded series of ethanol solutions. Tissues were then cleared with propylene oxide and embedded in epoxy resin (Embed 812, Electron Microscopy Science, Hatfield, PA). Semi-thin sections were cut at 2 μm with a Reichert ultramicrotome (Leica Microsystems, Wetzlar, Germany), and stained with toluidine blue for tissue verification. Ultra-thin sections were cut at 70 nm using a diamond knife (DiATOME diamond knives, Hatfield, PA) and placed on 200 mesh copper grids (Electron Microscopy Sciences, Hatfield, PA). Tissues were then stained with uranyl acetate and lead citrate. Specimens were viewed using a JEOL 100 transmission electron microscope, photographed with a L3C CCD digital camera (Scientific Instruments and Applications, Duluth, GA), and composite micrographs were constructed using Adobe Photoshop 7.0.

**Statistics Methods**

Ten measurements were taken of epididymal duct diameter (μm) and epithelial height (μm) from each individual within the proximal region of the epididymis. This region of the excurrent ducts is highly secretory, and to quantify seasonal variation of the secretory activity statistical tests were conducted. Sample size was low (e.g., one specimen per month). Therefore, data was nested to investigate significant differences between months, and then tested for seasonal differences (e.g.,
observed within the epididymal sheath (Fig. 2A, Ag). The ductuli efferentes merge into the apical portion of the epididymis (Fig. 2A, D, Ep) which consists of a pseudostratified columnar epithelium, although basal cells are rarely observed. All efferent ducts stain PAS+ and BB+ (Fig. 3) throughout the year with a lesser degree of intensity during October—December.

The nested ANOVA revealed no significant differences between seasons \((P = 0.15)\). However, measurements of epithelial height and tubule diameter with standard deviation for seasons (Fig. 4A) and months (Fig. 4B) are presented as these data mirror the secretory activity of the epididymis epithelium and indicate the changes that occur throughout the year. Measurements revealed that the spring \((130 \mu m \pm 35.60 \mu m)\) and summer \((141.27 \mu m \pm 9.99 \mu m)\) seasons have higher epididymis diameters than both the fall \((60.37 \mu m \pm 35.09 \mu m)\) and winter \((67.60 \mu m \pm 13.03 \mu m)\) seasons (see graph Fig. 4A).

**Rete Testis**

The rete testis (Fig. 2A, B, Rt) is composed of a simple squamous to low cuboidal epithelium surrounding a central lumen (Fig. 5A, B, Lu) where sperm (Fig. 5A, Sn and St) can be found during the spermatogenically active months. The nuclei (Fig. 5A, Nu) are irregularly shaped, usually bifurcated, heterochromatic, and basal. In the tunica propria, fibroblasts (Fig. 5A, Fn) and associated collagen fibers (Fig. 5A, Cf) form a sheath similar in diameter to that of the enclosed epithelium. Most rete testis epithelial cells possess short microvilli (Figs. 5B, 6A, Mv), and ciliated cells are sparse. When present, cilia (Fig. 5B, Ci) are confirmed by the presence of basal bodies (Fig. 5B, Bb) within the apices of the epithelial cells. Labyrinthine intercellular canaliculi are observed throughout the rete testis, and they often possess widened cavities (Figs. 5C, 6A, Ic). Some interruptions in the intercellular canaliculi occur, and these are associated with intracytoplasmic cavities containing debris (Fig. 6A, Db) and membranous structures (Fig. 6B, Ms). Basally, junctional complexes are absent and the intercellular canaliculi open directly into the tunica propria (Figs. 5C, 6C, Op). Elsewhere the intercellular canaliculi have multiple desmosomes (Figs. 5C, 6B, Ds) before being sealed by tight junctions (Fig. 5C, Tj) at the luminal border. Rarely, leukocytes, apparently monocyte-derived macrophages or T lymphocytes (Hermo and Robaire, 2002), occur in the basal portions of intercellular canaliculi (Fig. 6D, Lk). Small vesicles open into the intercellular canaliculi (Figs. 6C, Ve) and into the luminal (Fig. 7A, Ve) and basal (Fig. 7B, Ve) border, but such vesicles are not numerous. Lysosomes (Fig. 7B, C, D, Ly), and mitochondria (Fig. 7B, D, Mi) can be observed throughout the cytoplasm. Profiles of Golgi bodies (Fig. 7D, Go), and rough endoplasmic reticulum (Fig. 7D, Rer) can be found in supranuclear regions but are not numerous. No distinct secretory granules are present.

During the fall months the lumen of the rete testis is devoid of sperm (Fig. 8A, Lu). The epithelium still consists of both ciliated and scattered nonciliated cells (Fig. 8A, Ci). The collagenous sheath around the rete epithelium appears more irregular (Fig. 8A, C) than during the active months. Intercellular canaliculi (Fig. 8B, Ic) are generally less labyrinthine and narrower than in the spring and summer months. The only conspicuous organelles are mitochondria (Fig. 8C, Mi), some of which

**RESULTS**

**General Morphology and Seasonal Variation**

The testes lie dorsal to the liver suspended by the mesorchium and are composed of seminiferous tubules (Fig. 2, St), which are composed of a germinal epithelium and associated lumina. The seminiferous tubules converge at the anteriomedial aspect of the testis where a single tubule (Fig. 2, St), which are composed of a germinal epithelium and associated lumina. The seminiferous tubules converge at the anteriomedial aspect of the testis where a single tubule (Fig. 2, St) or an intratesticular rete testis (Fig. 2A, De) that consist of a ciliated simple short columnar epithelium. At the divergence of the rete testis to the ductuli efferentes the adrenal gland can be
are highly elongated, lysosomes (Fig. 8C, Ly; 8D, black arrowheads). Lysosomes appear more numerous than in the active months. Associated with the apical lysosomes are lipoidal bodies (Fig. 8C, Lb), which are not observed basally.

Ductuli Efferentes

The ductuli efferentes (Fig. 2A,C, De) are lined by a simple cuboidal epithelium that consists of both ciliated and nonciliated cells with ciliated cells being more numerous. As with the rete testis, the basal border is surrounded by collagen fibers (Fig. 9, Cf), but this sheath is relatively narrow compared to the width of the epithelium. The nuclei (Fig. 9, Nu) are large and heterochromatic with prominent nucleoli (Fig. 9, No). Long cilia (Figs. 9,10A, Ci) stretch into the middle of the lumen (Fig. 9, Lu) and contact cilia from the opposing side. Mitochondria (Fig. 9, Mi) and large vacuoles (Fig. 9, Va) are abundant in all cells. A well-developed brush border of microvilli (Fig. 9, Mv) originates from the apical portion of the nonciliated cells. Inter cellular

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Fig. 5. Electron micrograph of the rete testis. (A) Overview of the active rete testis showing the simple squamous epithelium surrounding the lumen (Lu) containing sperm (Sn; sperm nuclei, Sf; sperm flagellum). Multiple fibroblast nuclei (Fn) and associated collagen fibers (Cf) occur in the tunica propria. The epithelium contains irregularly shaped heterochromatic nuclei (Nu) and numerous mitochondria (Mi). Intercellular canalculus (Ic) Scale = 2 μm. (B) Enlarged view of the apical portion of an epithelial cell of the rete testis detailing the microvilli (Mv), cilia (Ci) and associated basal bodies (Bb). Basal lamina (Bl); Lumen (Lu). Scale = μm. (C) Enlarged view of an epithelial cell detailing the irregularly shaped nucleus (Nu). Intercellular canalculi (Ic) wind throughout the cytoplasm of the cell and are apically closed by desmosomes (Ds) and tight junctions (Tj). Basally the intercellular canalculi open (Op) into the basal lamina. Scale = 0.5 μm.
canaliculi (Fig. 10A,B,D, Ic) are narrow and not as highly interdigitated as those in the rete testis. Tight junctions (Fig. 10A, Tj) still appear at the luminal aspect, but desmosomes are not numerous. Occasional small vesicles (Fig. 10B, Ve) can be observed originating from the basal membrane and filamentous clusters of smooth endoplasmic reticulum (Fig. 10B, Ser) occur infranuclearly. Lysosomes (Fig. 10C,D, Ly), often associated with lipoid bodies (Fig. 10D, Lb), are scattered throughout nonciliated cells, sometimes in close association with intracytoplasmic spaces like those noted in the rete testis (Fig. 10D, Is). Rough endoplasmic reticulum (Fig. 10D, Rer) and cylindrical mitochondria (Fig. 10D, Mi) are also observed in close association with the lysosomes.

During the fall months the ductuli efferentes contain residual amounts of sperm (Fig. 11, Mpt and Sn) within the lumen (Fig. 11, Lu). Little seasonal variation occurs except for the decrease in tubule diameter. Cilia are well-developed, and ciliated cells (Fig. 11, Ci) are still more numerous than secretory cells. Apocrine secretions (Fig. 11, Ab) can be seen budding into the lumen as a bleb of secretory material. Intercellular canaliculi (Fig. 11, Ic) are narrow and straight (Fig. 11, Bl). Multiple lysosomes (Fig. 11, Ly) occur and evidence of spermioaphagic activity is observed with sperm nuclei (Fig. 11, Sn) and sperm tails (Fig. 11 inset, white arrowheads) embedded within the cytoplasm in close association to the secondary lysosomes.

**Ductus Epididymis**

The epididymis (Fig. 2A,D, Ep) lies within a relatively thin sheath of connective tissue and is comprised of a pseudostratified epithelium consisting of tall columnar secretory cells (principal cells) and basal cells (Fig. 12A, Bc). In active months, the basal nuclei (Fig. 12B, Nu) of principal cells are euchromatic with prominent nucleoli (Fig. 12B, No). The basal cells lie in close proximity to the basal lamina and are sparse. Electron-dense apical secretory granules (Fig. 12B, Sg) are numerous in principal cells during the active months. In addition to

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**Fig. 6.** Electron micrographs of the active rete testis. (A) Widened intercellular canaliculi (Ic) containing some debris (Db). Microvilli (Mv); Nucleus (Nu) Scale = 2 μm. (B) An interruption of an intercellular canaliculus (Ic) associated with a intracytoplasmic space (Is) containing membranous structures (Ms) and microfilaments (Mf). Desmosomes (Ds); Scale = 2 μm. (C) An intercellular canaliculus (Ic) detailing a vesicle (Ve) budding from the canaliculi and the opening (Op) into the tunica propria. Scale = 1 μm. (D) A leukocyte (Lk) within an intercellular canaliculus (Ic). Nucleus (Nu). Scale = 2 μm.
secretory granules, small clear vesicles (Fig. 13A, Ve) occur along the luminal border. Cilia are lacking in the epididymis, but short microvilli are numerous (Fig. 13A, Mv). Sperm in the lumen (Fig. 13A, Sp) are associated with a matrix of granulated material (Fig. 13A, Gm), presumably released from the secretory granules. The intercellular canaliculi are narrow apically (Fig. 13B, Ic), but basally contain large swellings (Fig. 13C,D, Ic), which often contain vacuolated material. Enlarged cisternae of rough endoplasmic reticulum (Fig. 13C,D, Rer) are abundant perinuclearly.

During the inactive months the epithelium is shortened and sperm are absent in the lumen (Fig. 14, Lu). The shrinking of the diameter of the epithelial cells causes bunching of the connective tissue sheath, which makes the basal border of the epididymis irregular. Residual amounts of secretory granules (Fig. 14B, Sg) and condensing granules (Fig. 14B, Cg) are observed at the luminal aspect of the epithelial cells, associated with narrow strands of rough endoplasmic reticulum (Fig. 14B, Rer). Lysosomes (Fig. 14A, Ly), some of which contain lipid bodies (Fig. 14A, Lb), are observed, but they are not as prominent as in the inactive ductuli efferentes. The intercellular canaliculi are sealed apically by tight junctions (Fig. 14B, Tj), possess numerous desmosomes (Fig. 14B, Ds), and are wider than those in active months. In some areas, the intercellular canaliculi are swollen apical to the basal cells (Fig. 14, Bc) and contain membranous structures (Fig. 14C, Ms) and filamentous material.

**DISCUSSION**

The overall morphology of the reproductive system in *H. turcicus* is similar to that of other reptiles (Siegel et al., 2009; Sever, 2010) and birds (Aire, 2007). The testes are suspended into the pleuropelvinocele from the mesenterium, and the epididymis, which is highly coiled, runs posteriorly (ventral to the
kidneys). A single tubule emerges from the external tunic of the testes, signifying the extra-testicular rete testis as described by Sever (2010) in his synonymizing of the terminology for reptiles. Although the number of extratesticular rete tubules varies (Table 1), the presence of a single rete tubule in H. turcicus is the same as in a diverse assemblage of lizards: Sitana ponticeriana (Akbarsha et al., 2006a), Eumeces fasciatus, (Reynolds, 1943), Lacerta vivipara (Martin-Saint-Ange, 1854), and Sceloporus undulatus (Forbes, 1941). Thus, this character may not have any phyletic significance. Instead, the authors suggest that the number of rete tubules may correspond to testicular size or length. Species with larger testes may contain more seminiferous tubules and therefore need more outlets to drain the tubules. Here we suggest that since only one rete tubule is observed only one or two seminiferous tubules make up the testis of H. turcicus. A longitudinal canal connecting the rete testis to the ductuli efferentes is not present. The presence of only one rete testis tubule does not correlate with presence or absence of a longitudinal canal.

Fig. 8. Electron micrographs of the inactive rete testis. (A) Overview of the rete testis showing the lumen (Lu) devoid of sperm and the epithelium consisting of ciliated (Ci) and nonciliated cells. The epithelium rests on a layer of irregular collagen fibers (Cf). Scale = 5 μm. (B) Enlarged view of the rete testis epithelium showing the narrow and relatively straight intercellular canaliculi (Ic) during inactive months with apical tight junctions (Tj) and desmosomes (Ds). The nuclei (Nu) contain a prominent nucleolus (No). Scale = 1 μm. (C) Apical portion of the rete testis epithelial cell detailing the elongated mitochondria (Mi) and lysosomes (Ly) containing lipid bodies (Lb). Scale = 0.5 μm. (D) Basal portion of the rete testis epithelial cell showing the basal lamina (Bl) and lysosomes (black arrowheads). Scale = 1 μm.
Although the nested ANOVA revealed no significant differences among months or seasons due to the high variation within the artificially created groupings and low sample size, some general conclusions can be drawn when adding in presence/absence of sperm within the testicular ducts. The testicular ducts of *H. turcicus* appear to have a single peak in activity, beginning November and December, and continuing to August. By September, activity levels decrease, entering a quiescent period in September to November suggesting a mixed reproductive cycle. The decrease in tubular diameter may be a direct result of spermiation. Rheubert et al. (2009) showed that the testes of *H. turcicus* have two peaks of spermiogenesis and therefore two major waves of spermiation. They reported that a wave of spermiation occurs in March, and this wave corresponds to the increase in efferent duct tubule diameters during that time. Their study also reported a second increase in spermiation (although spermiation continues between the two waves) in May-August during which an increase in the efferent duct morphometrics is also observed. The seasonal activity also corresponds with the results of Eckstut et al. (2009) who showed that the efferent ducts of *H. turcicus* contained residual amounts of sperm during the month of September and were devoid of sperm in October to December. Their study also showed that females contained sperm within the oviducts during the months of May-August which correlates with presence of sperm within the efferent ducts and when the efferent ducts are most active. This cycle is similar to other temperate squamates in which a peak in reproductive activity is observed during the warmer months of the year and a quiescent stage is entered in the late fall to early spring (Fitch, 1970). Although extreme variation in the number of rete testis tubules exists among squamates and other vertebrates, the structural morphology remains consistent. In amniotes the rete testis is composed of a simple squamous epithelium with bifurcated or irregular shaped nuclei and ciliated and nonciliated (principal) cells (Djakiew and Jones, 1981; Jones, 2002; Holmes and Gist, 2004; Aire, 2007; Akbarsha et al., 2007; Sever, 2010). Ciliated cells are not numerous in the rete testes of *H. turcicus* and more than one cilium projects into the lumen from a single cell unlike the ciliated rete cells described in mammals (Dym, 1976; Jones, 2002). Although this is the first ultrastructural study on the rete testis of a lizard, ultrastructural studies do exist on extend off nonciliated cells. The heterochromatic nuclei (Nu) possess prominent nucleoli (No). Collagen fibers (Cf) surround the basal portion of the ductuli efferentes. Mitochondria (Mi). Scale = 5 μm.

![Fig. 9. Overview of the active ductuli efferentes showing the ciliated and nonciliated cells. Cilia (Ci) extend into the lumen (Lu). Clear vacuoles (Va) can be seen within the lumen. Short microvilli (Mv) extend off nonciliated cells. The heterochromatic nuclei (Nu) possess prominent nucleoli (No). Collagen fibers (Cf) surround the basal portion of the ductuli efferentes. Mitochondria (Mi). Scale = 5 μm.](image)
other reptiles, including a snake (Sever, 2010), a turtle (Holmes and Gist, 2004) and a crocodilian (Guerrero et al. 2004). None of these other reptilian studies has reported cilia in the rete testis.

Intraepithelial leukocytes are present in the rete testis of *H. turcicus*, resembling the "halo cells" known from the mammalian epididymis (Hermo and Robaire, 2002) and reported in the epididymis of lizards by Meeran et al. (2001) in *Calotes versicolor* and Akbarsha et al. (2006b) in *Sitana ponticeriana*. Akbarsha et al. (2006a) correctly identified these halo cells as intraepithelial leukocytes, and Hermo and Robaire (2002) consider these leukocytes are either monocytes or T lymphocytes. We are unaware of other reports of intraepithelial leukocytes in the rete testis of vertebrates, and we did not encounter these cells in the ductuli efferentes or epididymis of *H. turcicus*.

Similar to other vertebrates, the epithelium of the rete testis of *H. turcicus* during the spermatogenically active periods is expanded with a stretched basal lamina and a tunica propria containing regular layers of collagen fibers, suggested to be components of the mediastinum testis (Leeson, 1962). During the spermatogenically inactive months the basal lamina becomes more scaffolded and the tunic of collagen fibers becomes more irregular. Contrary to mammals which exhibit continuous sperm release, reptiles release sperm in large bursts (Gribbins et al., 2003; Gribbins et al., 2008; Rheubert et al., 2009) and a high influx of spermatozoa and other spermatogenic components into the rete testis would cause the epithelium to stretch and push against the basal compartments.

Early descriptions of the rete testis suggested that it was merely a conduit for sperm, but more recent reports (within the past 20 yrs) have shown that the rete testis plays a major role in absorption of luminal fluids (Hermo et al., 1994) and possibly sperm maturation (Hess, 2002). The labyrinthine intercellular canaliculi are swollen during the active months, which suggest that water and water-soluble particles are being taken up by the

Fig. 10. Electron micrographs of the active ductuli efferentes. (A) Ciliated cell showing the cilia (Ci) in the lumen and a narrow intercellular canalculus (Ic) with apical tight junctions (Tj). Scale = 2 μm. (B) Enlarged view of the basal portion of a ciliated cell showing vesicles (Ve) budding from the basal membrane and smooth endoplasmic reticulum (Ser) basal to the nucleus. Basal lamina (Bl); intercellular canalculus (Ic). Scale = 1 μm. (C) Clusters of lysosomes (Ly) occur in both ciliated and nonciliated cells. Scale = 2 μm. (D) Supranuclear area of a nonciliated cell showing rough endoplasmic reticulum (Rer), an intercytoplasmic space, and scattered lysosomes (Ly) with lipid bodies (Lb). Intercellular canalculus (Ic); mitochondria (Mi). Scale = 1 μm.
epithelium (Hermo and De Melo, 1987). Tight junctions seal the apical aspects of the epithelium but do not occur basally, which may allow transport of materials externally through the intercellular canaliculi. Small vesicles are observed along the canaliculi as well as the basal membrane. Hermo and De Melo (1987) described a similar process for the movement of proteins through the epithelium into the stroma of the ductus deferens of the rat, and termed it transcytosis. The dilated intracytoplasmic spaces between cells are also suggestive of transport of water across epithelium (Hermo et al., 2002). During the inactive months sperm are absent and the widened, labyrinthine morphology of the intercellular canaliculi is lost.

Spermiophagic activity was not observed in the rete testis as described by Sinowatz et al. (1979) in bovine rete testis. Also, no secretory granules were observed in the rete testis although rough endoplasmic reticulum was observed. Hermo et al. (1994) proposed that rough endoplasmic reticulum plays a role in transforming endocytotic material.

The ductuli efferentes of H. turcicus are lined by a cuboidal epithelium that consists of ciliated and nonciliated cells consistent with reports in mammals (Ilio and Hess, 1994), birds (Aire, 2007), and other reptiles (Guerrero et al., 2004; Akbarsha et al., 2007; Sever, 2010). In addition to being a conduit for sperm, the ductuli efferentes have been ascribed roles in fluid resorption, secretion, and spermiophagy (Hess, 2002). The ductuli efferentes of vertebrates typically lack large secretory granules but contain apocrine blebs (Ilio and Hess, 1994) like we found in H. turcicus during the inactive months. These blebs may be responsible for the release of a granular secretion into the lumen (Sever, 2010). Intracytoplasmic spaces, as those in the rete testes, may function in water and solute transport. We found numerous lysosomes, especially in the inactive months, and evidence of spermiophagy, which is well-known in the ductuli efferentes of birds (Aire, 2007) and mammals (Hess, 2002) and was described by Akbarsha et al. (2007) in the lizard Sitana ponticeriana.

During the spermatogenically inactive months apocrine blebs are observed in the ductuli efferentes and the number of secondary lysosomes increases. Sperm can be found embedded within the cytoplasm which is consistent with spermiophagic activity described by Akbarsha et al. (2007) in the lizard Sitana ponticeriana. Since no
Fig. 12. Light microscopy of the active epididymis stained with hematoxylin and eosin. (A) Overview of a transverse section of the epididymis filled with sperm (Sp) detailing the columnar epithelial cells with rare basal cells (Bc). Scale = 25 μm. (B) Enlarged view of the columnar epithelial cells lining the epididymis with apical secretory granules (Sg). Nuclei (Nu) are euchromatic with prominent nucleoli (No). Sperm (Sp). Scale = 50 μm.

Fig. 13. Electron micrographs of the active epididymis. (A) Secretory cell of the epididymis detailing sperm (Sp) in lumen with copious amounts of granulated material (Gm). Small vesicles (Ve) are found at the luminal border and electron-dense secretory granules (Sg) are tightly packed. Microvilli (Mv); tight junctions (Tj). Scale = 2 μm. (B) The cytoplasm of a columnar cell between the luminal border and the nucleus with secretory granules in various stages of maturation. Note the narrow intercellular canaliculus (Ic) in this area. Scale = 1 μm. (C) Supranuclear area showing swollen areas of the intercellular canaliculi (Ic), and enlarged cisternae of rough endoplasmic reticulum (Rer). Mitochondria (Mi); nucleus (Nu). Scale = 1 μm. (D) Basal view showing continuation of the swollen intercellular canaliculi (Ic) and rough endoplasmic reticulum (Rer) alongside an euchromatic nucleus (Nu) with a prominent nucleolus (No). Basal lamina (Bl). Scale = 2 μm.
secretory activity was observed during the active months the apocrine secretion may be tagging the spermatozoa for phagocytosis.

The ductus epididymis consists of a pseudostratified columnar epithelium consisting of principal cells and basal cells consistent with other reports on the epididymis (see Hermo and Robaire 2002 for a review). Multiple reports on the epididymis typically recognize four different regions of the epididymis, the initial segment, caput, corpus, and cauda (Jones, 1998). However, no regional differences were observed along the length of the epididymis except for a gradual increase in size posteriorly which is similar to that described in Hemidactylus flaviviridis (Haider and Rai, 1981) and Tropidurus itambre (Ferreira et al., 2009; Table 2). The regions of the epididymis described in mammals and in the lizard Sitana ponticeriana are based on the presence or absence of six main cell types. In the lizard Sitana ponticeriana these cell types and their localized presence suggests four main regions similar to mammals but the regions were not found in H. turcicus (this study) or the snake Seminatrix pygaea (Sever, 2010). Only two cell types, principal and basal cells, were present in the epididymis of H. turcicus, which is the same as noted for Lacerta vivipara (Mesure et al., 1991), Podarcis sicula (Desantis et al., 2002), and S. pygaea (Sever, 2010).

During the active season the epididymis is highly secretory with apical secretory granules. The epididymis has been shown to be responsible for the synthesis and secretion of a wide range of proteins and glycoproteins (Dacheux and Dacheux, 2002; Hermo and Robaire 2002) and the activation and final maturation of spermatozoa (Acott and Hoskins, 1981). Contrary to the snake, Seminatrix pygaea, the secretory process of H. turcicus does not involve small vesicles and apocrine blebs but large electron-dense secretory granules similar to those of lacertids and agamids (Dufaure and Saint Girons, 1984). Mature secretory granules are uniformly electron-dense unlike the situation in Sitana ponticeriana, which has biphasic granules that release dense and less dense...
products separately (Akbarsha et al., 2006b). The secretion is both PAS- and BB+- similar to the histochemistry of other species (Manimekalai and Akbarsha, 1992; Labate et al., 1997; Sever, 2010). The epididymis is the first portion of the efferent ducts where the secretory material surrounding the sperm mass stains positively for proteins and carbohydrates. This suggests that only the epididymis secretes material that attaches to the sperm or that the sperm mass is not concentrated enough in other regions for the mass to stain positively during histochemical analysis.

During the inactive season of H. turcicus the epithe-
lium undergoes a major reduction in height due to the decrease in secretory activity. Interestingly, the intercellular canaliculi contain clusters of clear membranous structures and other debris apical to basal cells. The membranous structures have been hypothesized as being residuals of smooth endoplasmic reticulum activity (Hermo and De Mello 1987; Andonian and Hermo 1999). The presence of fibrous material within the multivesicular bodies suggests that the material may be spermatog- 

zoal degradation but no evidence for spermiophagic activity was observed within the epididymis such as we found in the ductuli efferentes of H. turcicus. Spermiophagy is well-known in the testes and efferent ducts of mammals (Jones, 2000) and birds (Aire, 2000). Akbarsha et al. (2006a,b; 2007) reported that the epithelium of both the “efferent ductules” and the cauda portion of the epididymis of Sitana ponticeriana is spermiophagic.

The reproductive system of H. turcicus resembles that of other vertebrates and amniotes and justifies the discarding of the terminology proposed by Volsæ (1944) and supports the synonymizing of the nomenclature suggested by Sever (2010). More research into the histology and ultrastructure of the reproductive system in nonmammalian vertebrates is needed to further elucidate the trends in the evolution of the reproductive system and its functions.

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