

Structural features of the epididymal region of the domestic duck (*Anas platyrhynchos*) Características estruturais da região epididimária do pato doméstico (*Anas platyrhynchos*)

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Abstract

The epididymal region of the domestic duck is composed by efferent ductules, whose histotopology was characterized by the proximal and distal ductules and sequentially by the epididymal duct. The epithelial lining of the efferent ductules was ciliated pseudostratified and formed by columnar cells. Also the ducts epididymidis epithelium was pseudostratified but non-ciliated. Concerning the histomorphometric analysis, the epithelial height mean was significantly greater in the distal efferent ductules, differing from the lower epithelium height mean observed in proximal efferent ductules and epididymal duct. The maximum and minimum diameter mean were significantly greater in the proximal efferent ductules, comparatively to the same diameter means of the other ductules.

Key-words:

Epididymal region.
Birds.
Morphology.
Morphometric analysis.

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Introduction

The seminiferous system of birds is constituted by two distinct portions, one intratesticular and other extratesticular. The intratesticular spermatic pathway is formed by seminiferous tubules and *rete testis* when present, which has an intratesticular portion and a tunical part.^{1,2,3,4,5} The extratesticular spermatic pathway is constituted by the extratesticular portion of the *rete testis*, by the epididymal region and *vas deferens*.^{6,7}

The extratesticular spermatic pathway is mainly characterized as pseudostratified stereociliated, with ciliated cells present in

the efferent ductules.^{5,6,8} In the *rete testis*, the epithelium is cuboidal simple, modified abruptly to pseudostratified epithelium in the efferent ductules, as observed in guinea fowl⁹ and in domestic dove.³

Morphologically, the epididymal region of birds consists of an interconnected tubular network of efferent ductules and epididymal duct.^{2,5} The efferent ductules might be histotopologically divided in proximal and distal, regard to the proximity or distance from the *rete testis*.^{6,10}

Morphometric analysis revealed that, in the epididymal region, the area occupied by proximal and distal efferent ductules

predominate in relation to the epididymal duct in the domestic rooster, Japanese quail, guinea fowl^{11,12} and domestic dove.^{3,5}

The extratesticular spermatogenic pathway has been studied by some authors^{3,4,5,6,7,11,13,14,15}, mainly in domestic birds. In these works, were observed that the physiological processes of sperm maturation, such as in mammals, occurred after the spermatozoa transit through the extratesticular seminiferous pathway.^{16,17}

On the basis of this information, the aim of this study was to analyze the histotopology of the epididymal region in the domestic duck (*Anas platyrhynchos*), as well as the structure of the tubular epithelia in this region at light and electron transmission microscopic levels, and also to analyze morphometrically the epithelial height, the maximum and minimum diameters of the proximal and distal efferent ductules and the same parameters to the epididymal duct.

Materials and Methods

Tissue fragments from the epididymal region were obtained from 8 adult domestic ducks (*Anas platyrhynchos*) during the active phase of the reproductive cycle, and examined by light and electron transmission microscopies. The ducks presented body weights between 3.0 and 3.5 kg, provided by creation farm in Jaboticabal, SP. The animals were killed by ethyl ether saturation (Sigma, USA), and then the epididymal region "in totum" was collected.

For histotopological analysis and examination of the tubular epithelium structure, fragments from the epididymal region were fixed in Bouin's solution and/or McDowell solution for 24 hours and included in Paraplast® (Oxford, Labware, USA) and Historesin® (Leica, Germany). Histological sections 5 to 3 µm thick were stained by Haematoxylin-eosin, Heidenhain Schleicher; and Haematoxylin-Plloxina B and 1% Toluidine Blue, and 0.5% Basic Fuchsin.

Fragments from the epididymal

region were fixed in Karnovsky's solution overnight and submitted to routine of electron transmission microscope. Ultrathin sections of 60-80nm were stained with uranyl acetate solution and lead citrate. The materials were examined and photographed in a Philips CEM-100 transmission electron microscope (Eindhoven, The Netherlands).

Maximum and minimum tubular diameters, epithelial heights of the epididymal duct, proximal and distal efferent ductules were measured in histological slides from 3 animals. Histomorphometric measures were obtained in 3 slides with 2 histological sections for each animal, totalizing 30 repetitions per animal, analyzed by means of the Zeiss KS 300 Computational Image-Analysis System (Carlzeiss, Germany). Data were submitted to analysis of variance (ANOVA) and the 5% Tukey Test, utilizing S.A.S. software (Statistical Analysis System, USA).

Results

The epididymal region of the domestic duck was formed by the proximal and distal efferent ductules and also by the epididymal duct, having as histotopological references their proximity or distance from the testicular network complex (Figure 1).

The proximal efferent ductules were lined by the pseudostratified columnar epithelium with ciliated and non-ciliated cells, and showed longitudinal folds which penetrate the tubular lumen (Figure 3). Ciliated cells presented nuclei of several forms, located basally or apically with cilia in the apical "brush border" (Figures 3, 8, 9). Non-ciliated cells possessed basal spherical nuclei and apical microvilli (Figure 8).

In the distal efferent ductules, epithelial cells showed abundant cilia in the apical region (Figure 4). Occasionally, small longitudinal folds were seen in the epithelium. Nuclei of the columnar cells were generally oval, large, and basally located, but some apical nuclei were observed (Figure 4).

Table 1

Means (\pm SEM) of the maximum and minimum diameters (mm) of proximal and distal efferent ductules and epididymal duct of the domestic duck.

	Proximal efferent	Distal efferent	Epididymal duct	Coefficient of Variation
Maximum diameter (μ m)	329.950 \pm 5.42a	81.036 \pm 3.99b	67.033 \pm 3.99c	0.82
Minimum diameter (μ m)	140.700 \pm 5.32a	67.116 \pm 4.55b	57.313 \pm 4.56c	2.60

* Different letters in the same line indicate a significant difference by the 5% Tukey Test

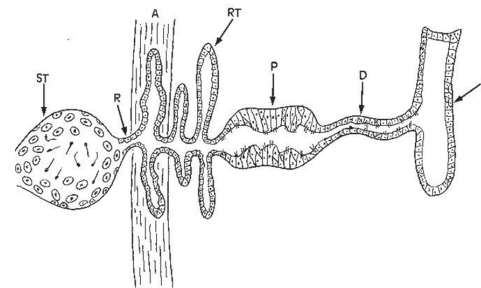


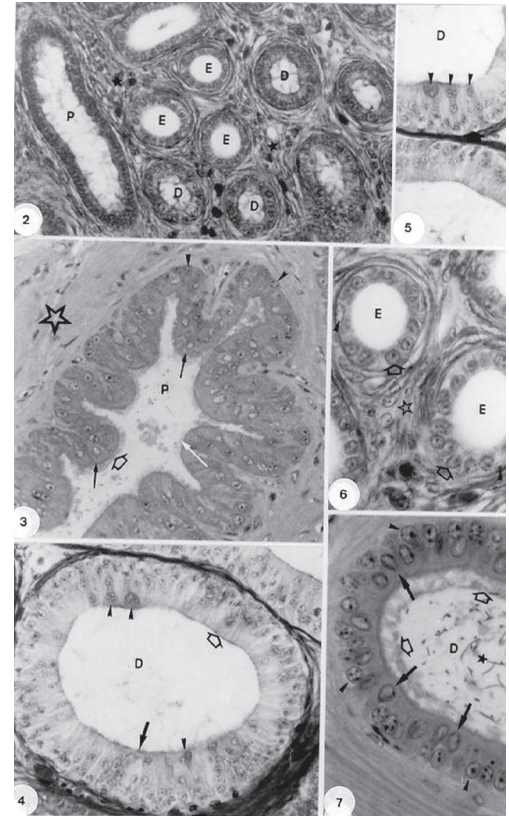
Figure 1

Schematic drawn of the epididymal region of the domestic duck. ST = Seminiferous tubules; R = recti tubule; A = tunica albuginea; RT = rete testis; P = proximal efferent ductule; D = distal efferent ductule; E = epididymal duct

Proximal and distal efferent ductules were surrounded by abundant connective tissue (Figures 2, 6) and the tubular lumen contained spermatozoa and cellular exfoliation from the preceding spermatid pathway (Figures 3, 4).

Sequentially, the distal efferent ductules join with the epididymal duct, which was short and presented a regular contour, without longitudinal folds (Figures 2, 3). The epididymis epithelium was pseudostratified columnar, showing occasional basal cells and absence of apical cilia (Figure 5). The spherical nuclei of columnar epididymal cells presented one or two evident nucleoli. Nuclei of basal cells were elongated with nucleoli located near the nuclear membrane (Figure 4).

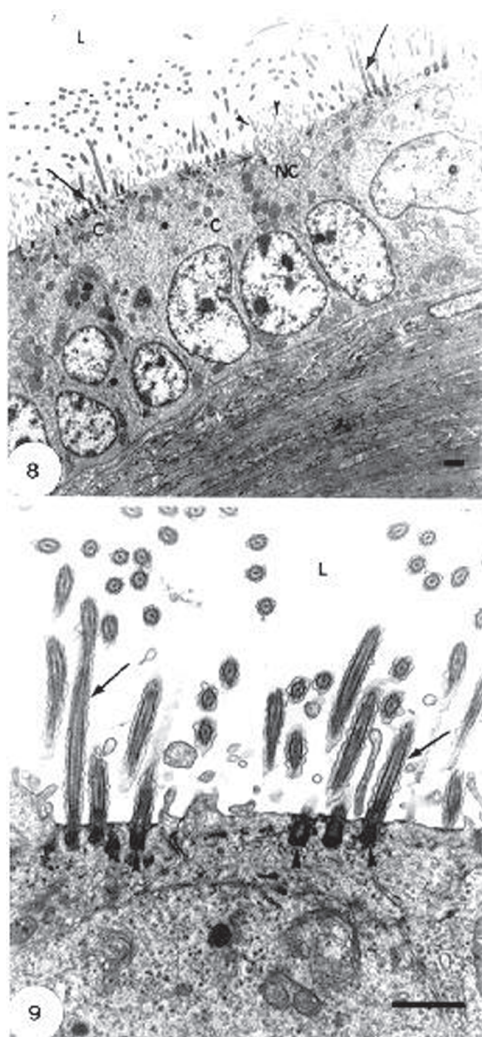
Morphometric analysis revealed that the proximal and distal efferent ductules occupied a larger area in the epididymal region of the domestic duck. The epithelial



Figures 2-7

Figure 2 - Epididymal region with the proximal efferent ductule (P), distal efferent ductule (D) and epididymal duct (E). Note the abundant connective tissue (stars) surrounding the ductules (x 100); Figure 3 - Proximal efferent ductule (P), with folding of the epithelium (arrow), ciliated pseudostratified epithelium formed by columnar cells (large arrow heads), non-ciliated cells (small arrow heads), cilia (white arrow) and surrounding connective tissue (star) (x 200); Figure 4 - Distal efferent ductule (D) showing pseudostratified ciliated epithelium (arrow) and columnar cells (large arrow head) with some apical nuclei (small arrow heads) (x 100); Figure 5 - Distal efferent ductule (D) with pseudostratified ciliated epithelium, basal (arrow) and apical (arrow head) cellular nuclei (x 100); Figure 6 - Epididymal duct (E) formed by the pseudostratified columnar epithelium, with occasional basal cells (arrow heads) and columnar cells (arrows); connective tissue (star) (x 200); Figure 7 - Detail of the epithelium of the distal efferent ductule (D), with pseudostratified epithelium forming a "border brush" (arrows). Nuclei of columnar basal cells (small arrow heads) with some apical (large arrow heads). Lumen with spermatozoa and exfoliated cells (400x)

height of the distal efferent ductules presented higher mean values (19.19 mm \pm 2.52), differing significantly from the proximal efferent ductules and the epididymal duct (Figure 10). However, epithelial height of the proximal efferent ductules (9.97 mm \pm 1.38) and of the epididymal duct (13.55 mm \pm 0.96) did not



Figures 8-9
 Epithelial lining of the proximal efferent ductule, showing non-ciliated cell (NC) with microvilli (arrow head) and ciliated cells (C) with their cilia (arrows); Tubular lumen (L). Scale bar = 1mm; Figure 9 Detail of the epithelial surface of the proximal efferent ductule, with cilia (arrow), basal corpuscle (arrow head) and tubular lumen (L). Scale bar = 1mm

differ significantly, presenting the smallest mean values (Figure 10).

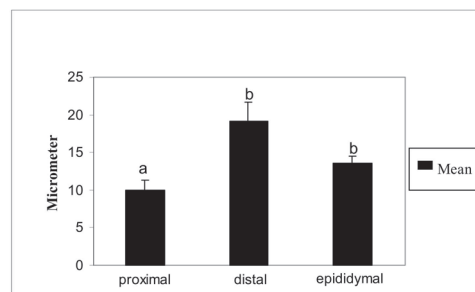
The maximum and minimum diameters of the distal and proximal efferent ductules as well as that of the epididymal duct differed significantly among themselves (Table 1). The maximum diameter of the proximal efferent ductules ($329.95 \text{ mm} \pm 5.42$) and the minimum diameter ($140.7 \text{ mm} \pm 5.32$) presented the

greatest mean values (Table 1). The maximum diameter of the distal efferent ductules ($81.03 \text{ mm} \pm 3.99$) and the minimum diameter ($67.11 \text{ mm} \pm 4.55$) showed intermediate values (Table 1). Yet the maximum diameter of the epididymal duct ($67.03 \text{ mm} \pm 3.99$) and the minimum diameter ($57.31 \text{ mm} \pm 4.56$) showed the smallest mean values observed (Table 1).

Discussion

The epididymal region of the domestic duck was formed by efferent ductules which could be characterized histotopologically in proximal and distal and by the epididymal duct, similar to that found in domestic rooster², turkey¹⁸ and domestic dove⁵, except for some species-specific differences.

Electron transmission microscopy and light microscopy analysis revealed a ciliated pseudostratified epithelium for the proximal and distal efferent ductules, being formed by columnar cells. The presence of intense longitudinal folds was observed in the epithelium of the proximal efferent ductules, forming a brush border that penetrate into the lumen. These ductules presented a shorter epithelial height, although a larger tubular diameter was seen comparatively to the distal efferent ductules and epididymal ducts. Theoretically, the efferent ductules could absorb a larger quantity of fluid from the testicular region. According to Aire¹³, the



* Different letters indicate a significant difference by the 5% Tukey Test

Figure 10
 Epithelial height means (mm) of the proximal, distal efferent ductules and epididymal duct

presence of epithelial folds, a wide lumen in the proximal efferent ductules and the larger concentration of spermatozoa in the distal efferent ductules observed in several species of birds and also in the domestic duck, support the hypothesis of absorptive role to the efferent ductules.

In the distal efferent ductules, the epithelial folding was discrete, having the same type of epithelium, greater epithelial height compared to the proximal efferent ductules and epididymal duct, but with cells showing abundant cilia. These characteristics of the efferent ductular epithelium as a whole have been observed in the domestic dove⁵, guinea fowl¹¹, turkey¹⁸ and Japanese quail¹⁹.

It is believed that the efferent ductules of birds are involved in physiology, maturation and metabolism of spermatozoa, and in maintenance of the intraluminal micro-environment of the extratesticular seminal pathway^{5,15,17,20}, due to the absence of accessory sex glands. The principal cytophysiological attribute of the spermatic ducts in birds was related to the maturation of spermatozoa, classically described for the mammalian epididymis²⁰.

The epididymal duct of the domestic duck is short, with a small diameter and regular contour, presenting a non-ciliated pseudostratified epithelium without folds, similar to those observed in domestic rooster¹⁴ and the domestic dove³. The tubular diameter of the epididymal duct was greater compared to the distal efferent

ductule of the domestic duck.

In the epididymal region of the rooster, Japanese quail, guinea fowl¹¹ and domestic dove⁵ there is a significant predominance of efferent ductules distribution. Regarding to the observations in the duck it was concluded that the epididymal region of this species was formed predominantly by the efferent ductules, having only a small contribution of the epididymal duct. However, Hess, Thurston and Biellier et al.¹⁸ observed a larger area occupied by the epididymal duct and a smaller area for the efferent ductules in the turkey differing from some bird species, including the domestic duck.

In agreement with our observations, the modest contribution of the epididymal duct in the epididymal region of the species studied, also may be related to its small role in the metabolism and maturation of spermatozoa, when compared to the epididymis of mammals according to Clulow and Jones^{16,21} and Howarth.¹⁷

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Resumo

A região epididimária do pato doméstico era composta pelos ductúlos eferentes, os quais hitotologicamente foram caracterizados como ductúlos eferentes proximal e distal e, sequencialmente, pelo ducto epididimal. O epitélio dos ductúlos eferentes era pseudo-estratificado, formado por células colunares. O epitélio dos ductos epididimários mostrou-se também pseudo-estratificado, mas não ciliado. De acordo com as análises histomorfométricas, a média da altura epitelial foi significativamente maior nos ductúlos eferentes distais, diferindo das baixas médias de altura epitelial observadas nos ductúlos eferentes proximais e ducto epididimal. A média dos diâmetros máximos e mínimos foi significativamente maior nos ductúlos eferentes

Palavras-chave:

Região epididimária.
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proximais, comparativamente as médias dos mesmos diâmetros dos outros ductulos.

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