Ultrastructural study of the luminal surface of the ducts of the epididymis of gallinaceous birds

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ABSTRACT

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The various ducts of the epididymides of four gallinaceous birds, the turkey (*Meleagris gallopavo*), domestic fowl (*Gallus gallus*), guinea-fowl *(Numida meleagris)* and Japanese quail (*Coturnix coturnix japonica*) were studied at the scanning and transmission electron microscopy levels. The tissues were fixed either by immersion or vascular perfusion, for comparative purposes. Each duct system, save for a few details, presented similar morphological features in all species. The epithelial surface of the rete testis was regular and each cell bore a single cilium, as well as numerous, or in some parts, very few, short, regular microvilli. Each of the Types I and II non-ciliated cells of the proximal and efferent ducts displayed abundant, moderately long and regular microvilli, and a solitary cilium. The ciliated cells exhibited tufts of cilia. The Type III non-ciliated cell of the connecting and epididymal ducts exhibited a solitary cilium, and numerous microvilli which were intermediate in length between those of the rete testis and those of the efferent ducts. Vascular perfusion of the avian epididymal tissue was the superior method of fixation because it minimised the developments of fixation artefacts. Apocrine secretion did not appear to occur in the epididymis of these birds as the apical blebs of Types I, II and III cells, which have previously been reported, only manifest in this study in inadequately fixed tissues, and were therefore viewed as being artefacts. The present findings suggest that the current terminology, as applied to the avian epididymis, be retained.

Keywords: Ducts, epididymis, gallinaceous birds, SEM and TEM

INTRODUCTION

The avian epididymis is a complex organ comprising several duct systems (Lake 1957; Tingari 1971; Budras & Sauer 1975; Hess, Thurston & Biellier 1976; Aire 1979a; Aire, Ayeni & Olowookorun 1979). The comparative anatomy of the epididymis of birds seems to vary considerably between species (Budras & Meier 1981), and the functions of the avian male genital ducts are not well understood (Clulow & Jones 1988). The varying interpretation(s) of structure and the possible functions of the epithelial cells lining the various ducts of the avian epididymis have therefore complicated an adequate understanding of the biology of these ducts.

The structure of the luminal surfaces of the various avian epididymal ducts has received scant attention, and this feature has only been studied in the domestic fowl and turkey (Bakst 1980), duck (Aire 1982a) and ostrich (Aire & Soley 2000). It is clear from these reports that there are significant differences in structural presentation and therefore in interpretation of the possible functions of the epithelial cells of these species.

In this study the topographical features of the epithelial lining of the rete testis (not included in the papers by Bakst 1980; Aire 1982a), the efferent ducts and the connecting and epididymal ducts of the epididymis of four members of the order Galliformes were examined and compared. This study also compares the structural features of the ducts between tissues fixed by vascular perfusion and immersion-fixed tissues.

MATERIALS AND METHODS

Five sexually mature and active male turkeys (*Meleagris gallopavo*), seven domestic fowls (*Gallus gallus*), ten Japanese quails (*Coturnix coturnix japonica*) and three guinea-fowls (*Numida meleagris*) were used in this study. The turkeys were obtained from a local farm, and the domestic fowls and quails were bought from the Animal Improvement Centre of the Agricultural Research Council, Irene, near Pretoria. Two of the guinea-fowls were kindly donated by Dr N.R. Bryson and the third was captured in the wild, under a permit. All birds were housed and maintained in the animal holding facility of the Medical University of Southern Africa, Medunsa, for at least one week before sacrifice.

Heparin, at a dosage of between 250 and 1000 i.u, depending on the size of the bird, was administered intramuscularly to each bird 15–30 min before sacrifice. Two fixatives and two fixation methods were employed. Two turkeys, three domestic fowls, four quails and two guinea-fowls were intravascularly perfused with 3% glutaraldehyde buffered in 0,067M cacodylate buffer, at pH 7,2, as previously described (Aire 1982a). Two turkeys, two domestic fowls and three quails were similarly perfused with 2,5% Karnovsky's fixative. In the turkey, domestic fowl and guinea-fowl, perfusion was performed via the descending aorta. Due to their size, the quails were perfused via the left ventricle of the heart by means of a needle introduced through the ventricular wall. The remaining birds (one turkey, two fowls, three quails and one guinea-fowl) were deeply anaesthetized by intraperitoneal injection of pentobarbitone sodium (Abbot Laboratories) at a dosage of 70 mg/kg body mass, but for the quails, chloroform inhalation anaesthesia was used. Tissues from the testis and epididymis were quickly fixed by immersion in glutaraldehyde or Karnovsky's fluid of identical composition to that used for the vascularly-perfused birds. Fixed epididymal tissue including some adjoining testicular tissue, and epididymal tissue alone, were respectively prepared for scanning (SEM) and transmission (TEM) electron microscopy, using standard techniques. The SEM samples were viewed in a Philips XL 20 scanning electron microscope, while the sections were studied in a Philips 301 or Jeol 100 CX transmission electron microscope.

RESULTS AND OBSERVATIONS

The general structure and organisation of the epididymis was observed to be similar in all four species studied, and was composed of a rete testis, proximal and distal efferent ducts, connecting and the epididymal duct (Fig. 1). Therefore, the surface features of each of the different duct types are described collectively for all species and any specific differences highlighted. As was expected, there were no differences due to the fixative used, but there was a pronounced difference in the appearance of some cells between the tissues fixed by vascular perfusion and those fixed by immersion. These differences are also highlighted. The terminology used to describe the various ducts and cell types follows that of Aire (1979a, 1980).

The rete testis

The rete lacunae, with their rete chords and mucosal folds, were lined by a relatively even epithelium (Fig. 2). The luminal surfaces of the cells usually appeared as elongated polygons (Fig. 3) although more rounded polygons were also frequently seen. The cell outlines appeared more sharply demarcated in the domestic fowl, guinea-fowl and quail than in the turkey. Stubby microvilli (measuring between 0,3 and 0,4 µm in length, in all birds) regularly marked the cell borders, and others were scattered irregularly and to varying extents throughout the surface of the cell. The number of microvilli varied considerably between cells in all species; a few cells displayed very few or no surface microvilli outside the borders (Fig. 4), but the surfaces of other cells displayed numerous or moderately abundant microvilli, either centrally (Fig. 3) or uniformly (Fig. 5 and 6). This was generally the case in the turkey (Fig. 6). A single cilium usually projected from the central region of most rete cells (Fig. 4 and 5). Luminal macrophages could be seen resting on the rete epithelium (Fig. 7). They were commonly observed in the domestic fowl, but much less frequently in the other species. The outlines of these cells were variable, and usually displayed cytoplasmic extensions or pseudopodia. Their cytoplasm contained irregular nuclei, dense bodies, probably lysosomes, and lipid droplets which were scattered throughout the cytoplasm (Fig. 7). They occasionally contained ingested spermatozoa.

The efferent ducts

• *The proximal efferent duct (PED)* displayed a highly folded mucosa in all species (Fig. 8). The folds projected prominently into the ductal lumen, thus considerably reducing the available luminal space in this duct in some cases. Ciliated and non-ciliated (Type I) cells composed the epithelium of this duct (Fig. 9, 10 and 11). The non-ciliated cells were more numerous than the ciliated cells, but the cilia
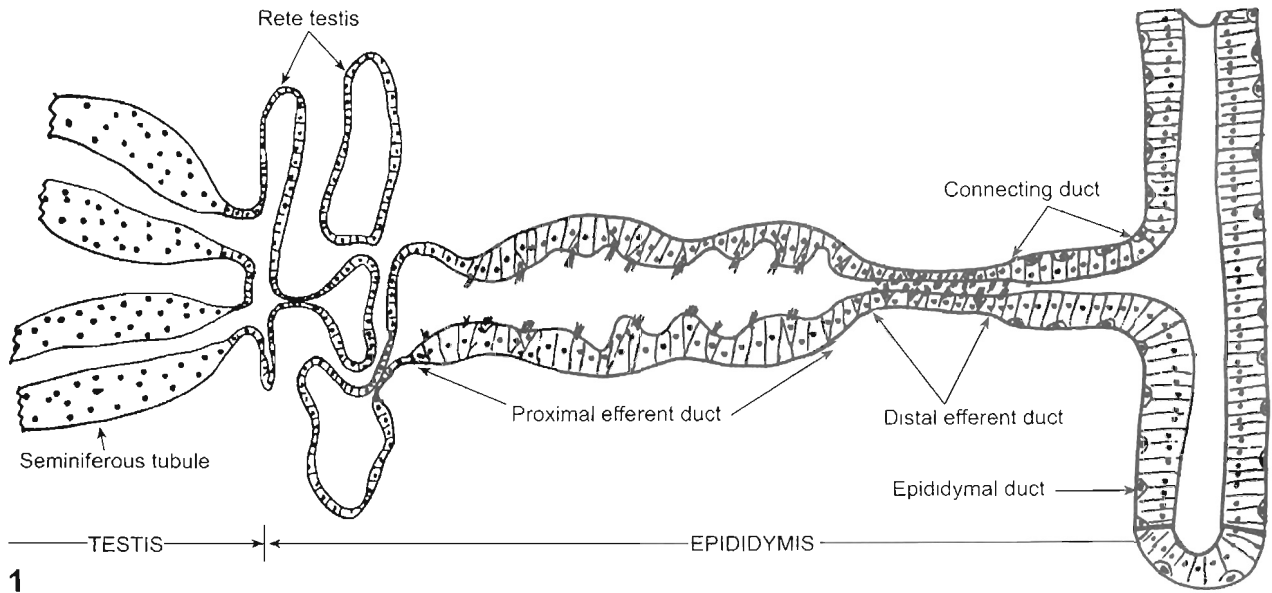
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FIG. 1 A schematic representation of the various ducts of the avian epididymis. Not drawn to scale
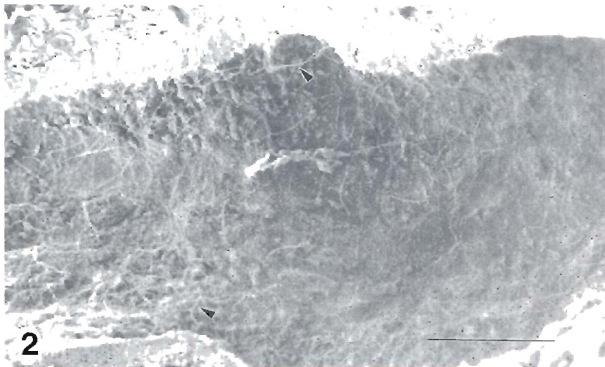

FIG. 2 A survey SEM micrograph of the luminal surface of the rete testis of the turkey. The epithelial surface is regular, and cilia (arrowheads) are visible
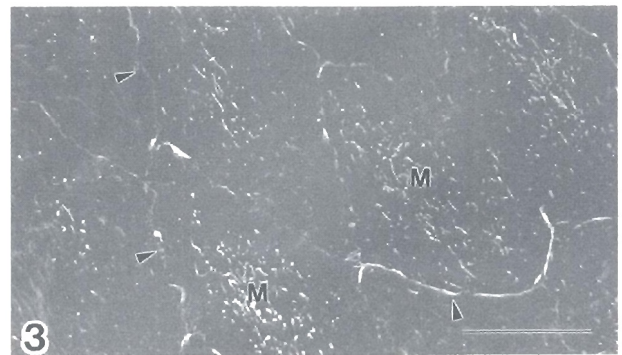
Bar = 20 µm. Perfusion fixation

FIG. 3 A survey SEM micrograph of the surface of the rete testis of the Japanese quail. The cell outlines (arrowheads) are mostly elongated polygons. M, numerous centrally-located microvilli
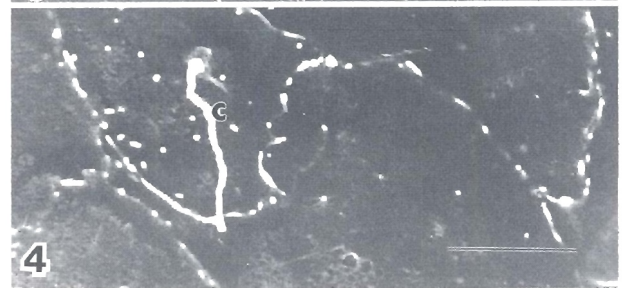
Bar = 10 µm. Perfusion fixation

FIG. 4 Sparse, stubby microvilli on some rete cells of the domestic fowl. C, cilium
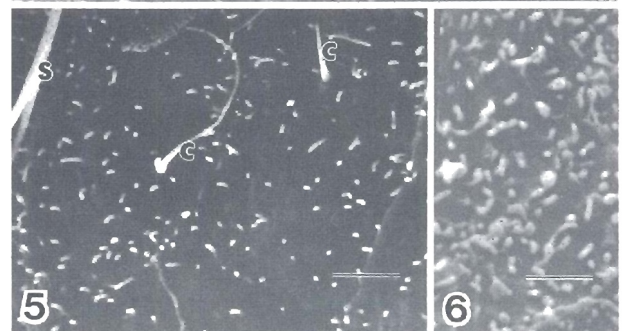
Bar = 2 µm. Perfusion fixation

FIG. 5 Rete cells of the domestic fowl illustrating moderately numerous, scattered microvilli. C, cilium; S, part of a spermatozoon

Bar = 1 µm. Perfusion fixation

FIG. 6 The luminal surfaces of the rete cells of the turkey illustrating abundant, evenly scattered microvilli
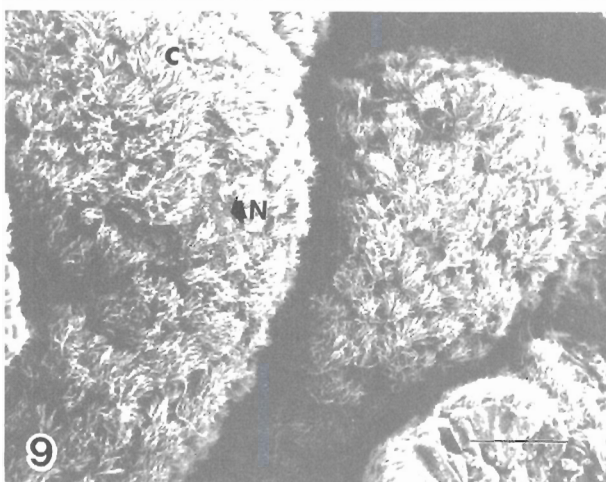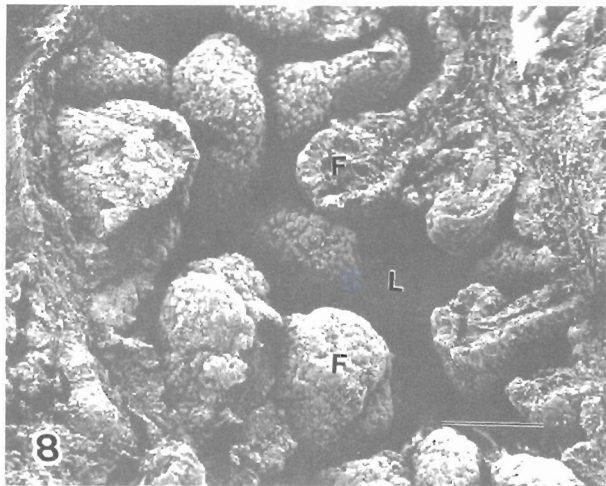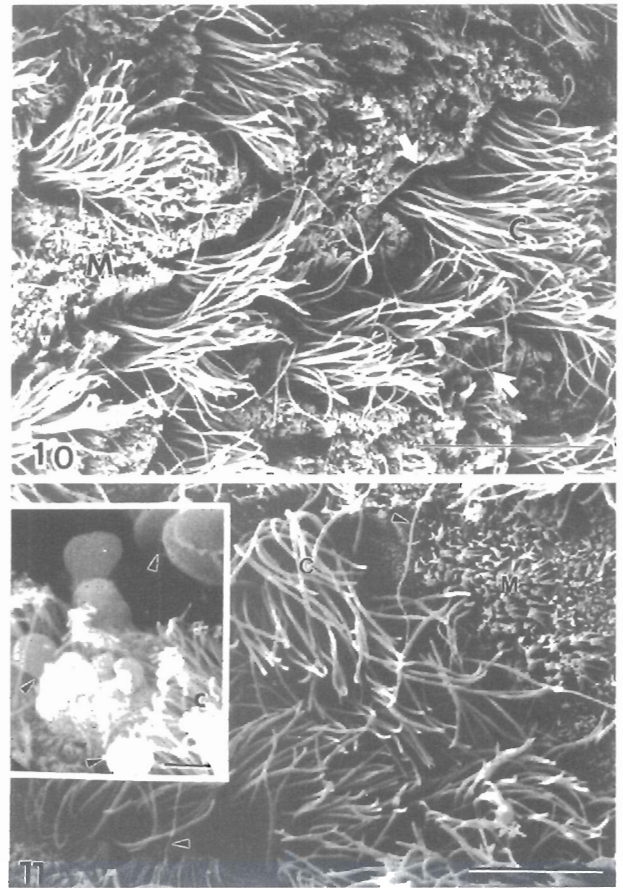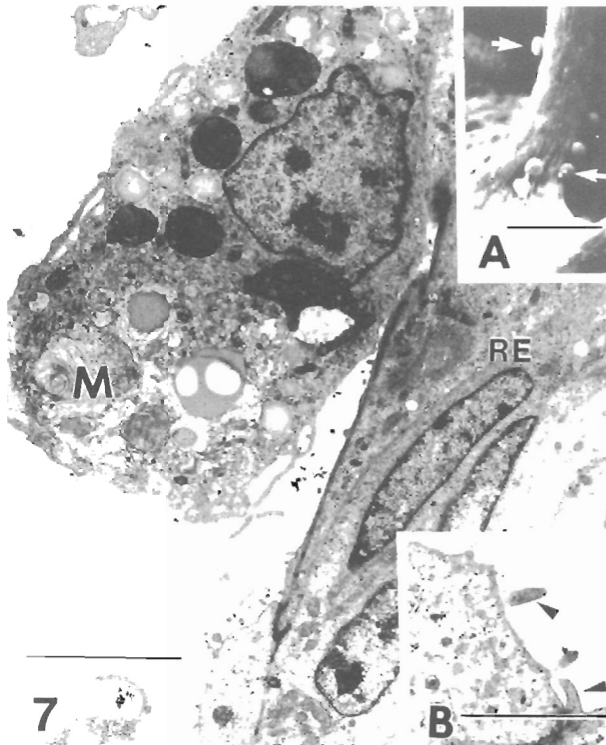
Bar = 1 µm. Perfusion fixation

FIG. 7   A luminal macrophage (M) rests on the rete epithelium (RE) of the domestic fowl. Bar = 5 µm

Inset A shows macrophages (arrows) lying on the rete epithelium of a *chordae retis*   Bar = 25 µm

Inset B shows the stubby microvilli (arrowheads) of the rete cells.

Bar = 1 µm Perfusion fixation

FIG. 8   A survey SEM micrograph of the epithelium of the proximal efferent duct (PED) of the domestic fowl. Note the mucosal folds (F) which fill the lumen (L) of the duct

Bar = 100 µm Perfusion fixation

FIG. 9   A higher power survey micrograph of the PED of the domestic fowl Ciliated cells (C) and non-ciliated (Type I) cells (N) are shown

Bar = 25 µm. Perfusion fixation

FIG. 10   A high power view of the PED of the Japanese quail. C, cilia of ciliated cells; M, microvilli of the non-ciliated cells. Arrow, single cilium of a non-ciliated cell

Bar = 10 µm Perfusion fixation

FIG 11   The microvilli (M) of the non-ciliated cells of the PED of the turkey are relatively shorter than those of the other birds. C, cilia of ciliated cells; arrowheads, solitary cilium of the non-ciliated cell

Bar = 5 µm. Perfusion fixation

Inset shows apical cytoplasmic blebs of non-ciliated cells in inadequately-fixed tissue. C, cilia of ciliated cells

Bar = 40 µm. Immersion fixation

of the latter usually over-shadowed the luminal surfaces of non-ciliated cells (Fig. 9 and 11). The ciliated cell projected a well-formed tuft of cilia (measuring about 5 μm in length in the domestic fowl, 7 μm in the guinea-fowl and quail, and 8 μm in the turkey) into the lumen of the duct, whereas the non-ciliated cells bore closely-packed, long and regular microvilli. The ciliated cells also bore microvilli which were scattered between the cilia, but they were, however, fewer, shorter and thinner than those of the non-ciliated cells (Fig. 12).

The microvilli of the non-ciliated cells in the turkey were markedly shorter (about 1,1 μm in length) than those in the other birds which, due to their length (about 2,5 μm), might be described as stereocilia (Fig. 10 and 11). In poorly-fixed tissue the luminal surfaces of several to most Type I cells tended to form cytoplasmic blebs which projected into the ductal lumen. In such tissue the microvilli of these cells also tended to become swollen or pleomorphic in shape (Fig. 16). In well-fixed, usually perfusion-fixed, tissue apical cytoplasmic blebs were only occasionally observed in the non-ciliated cells. The luminal plasmalemma of well-fixed Type I cells was regular, and extended into microvilli which were long and regularly cylindrical in shape

(Fig. 12). The subapical cytoplasm of the cells exhibited numerous tubular coated pits, apical tubules and vacuoles, as well as numerous dense bodies in the supranuclear zone (Fig. 12). A single cilium, in addition to the microvilli, projected into the ductal lumen from several, but not all, non-ciliated cells (Fig. 10 and 11).

• *The distal efferent duct (DED)* is a direct continuation of the proximal efferent duct (Fig. 1). In this study, its epithelial lining was usually regular and surrounded a small lumen which contained a plug of spermatozoa. The epithelium was also composed of non-ciliated (Type II) and ciliated cells (Fig. 13). The latter ciliated cells were, however, more numerous than the non-ciliated cells in this duct, and their cilia tended to conceal the luminal surfaces of the fewer non-ciliated cells. The Type II cells bore microvilli which were similar to those of the Type I cells of the proximal efferent duct. The SEM features of the two epithelial cell types found in this duct were similar to those of their respective counterparts in the proximal efferent duct, but the TEM cytoplasmic organelle configurations of Types I and II cells, but not the ciliated cells, were different (Fig. 14). The subapical cytoplasm of the Type II cells of the DED did not possess the elabo-
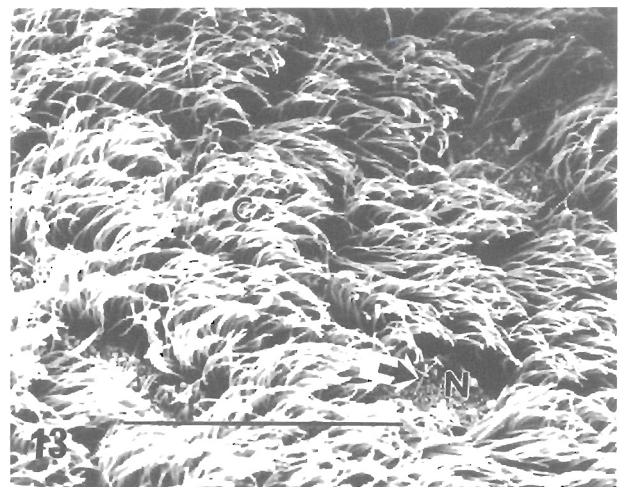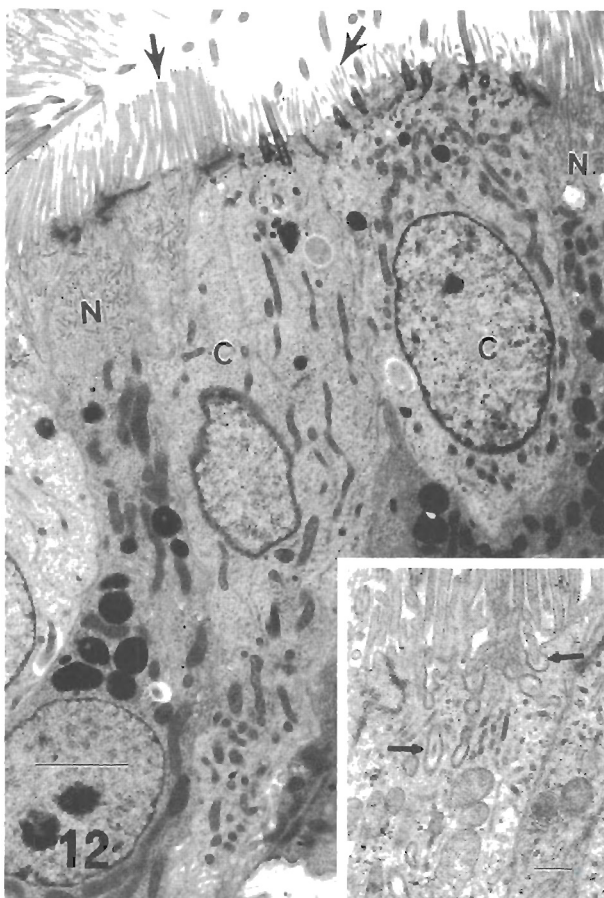

FIG. 12 A TEM micrograph of the PED epithelium of the domestic fowl showing ciliated (C) and non-ciliated (N) cells. The microvilli (arrows) are long and regularly cylindrical in shape in both cell types, but those of the ciliated cell are shorter and thinner

Bar = 1 μm

Inset shows the coated tubular pits and apical tubules (arrows) in the subapical zone of a non-ciliated cell

Bar = 1 μm. Perfusion fixation

FIG. 13 A SEM micrograph of the luminal surface of the distal efferent duct (DED) of the turkey. showing ciliated (C), and non-ciliated (N) cells. Arrow. single cilium of a non-ciliated cell
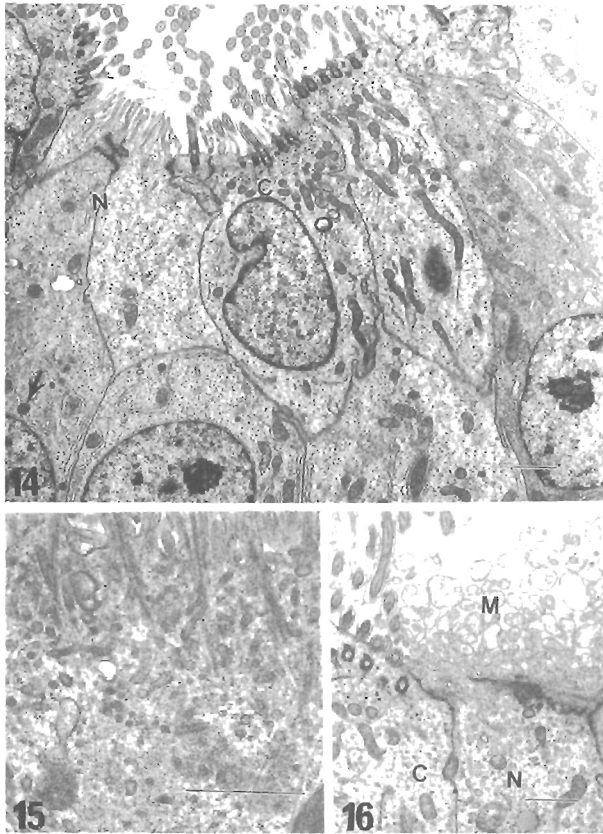
Bar = 20 μm. Perfusion fixation

FIG. 14   A survey TEM micrograph of the epithelium of the DED of the domestic fowl. Very few and small dense bodies (arrow) occur in the supranuclear region of the non-ciliated (Type II) cell (N). C, ciliated cell

Bar = 1 µm. Perfusion fixation

FIG. 15   The subapical region of the non-ciliated (Type II) cell of the DED contains only a few coated pits, apical tubules and vacuoles, and there are no dense bodies

Bar = 1 µm. Perfusion fixation

FIG. 16   The microvilli (M) of the non-ciliated (Type II) cell (N) are pleomorphic in improperly fixed tissue of the guinea-fowl. C, ciliated cell
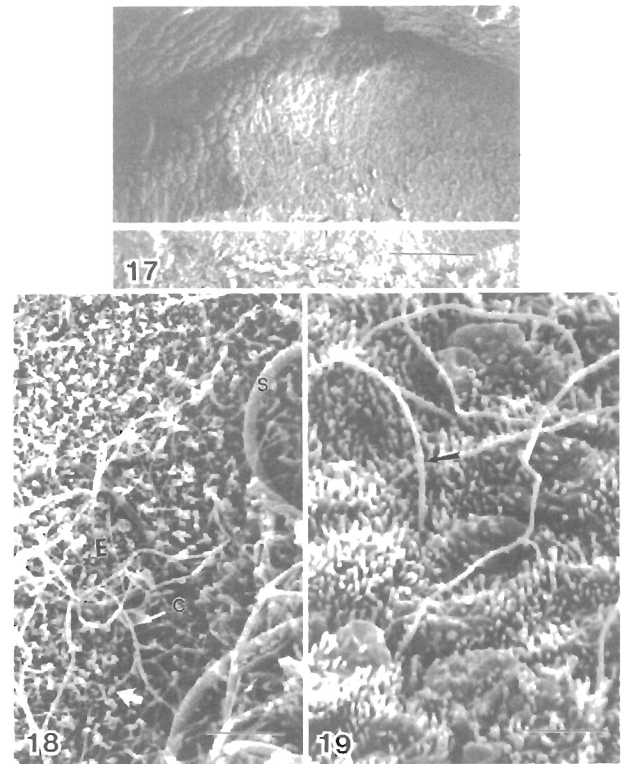
Bar = 1 µm. Immersion fixation

FIG. 17   A survey SEM micrograph of the epididymal duct of the turkey. The epithelial cell outlines are polygonal, especially on the left of the micrograph

Bar = 20 µm. Perfusion fixation

FIG. 18   A SEM micrograph of the epididymal duct of the Japanese quail. A few microvilli show flattened tips (arrows) resulting from the pressure of the plug of spermatozoa generally present in the duct. C, cilia of non-ciliated (Type III) cells; S, spermatozoon

Bar = 5 µm. Perfusion fixation

FIG. 19   The surface of the epididymal duct of the turkey shows microvilli and a single cilium (arrow)

Bar = 2 µm. Perfusion fixation

rate coated pits, apical tubules or dilated vacuoles which were found in the Type I cells of the PED. There were also very few or no dense bodies in the supranuclear region of the Type II cells (Fig. 12 and 14). A single cilium also generally projected from the non-ciliated cells, as in the proximal efferent duct. Apical cytoplasmic blebs were, again, rarely observed in tissues that were properly perfusion-fixed, but were common in immersion-fixed or improperly perfused tissues (Fig. 16).

The connecting duct and ductus epididymidis

The connecting duct links the distal efferent duct with the ductus epididymidis (Fig. 1). Its diameter and

epithelial height were less than those of the epididymal duct. However, both ducts were lined by an identical epithelium, in terms of apical or luminal surface features and organelle content. Both ducts are therefore described together.

The connecting and epididymal ducts were lined by an epithelium which was thrown into irregular grooves and a few low folds (Fig. 17). Mucosal folds were infrequently encountered. The principal epithelial cell of these ducts was the non-ciliated (Type III) cell. The borders between cells were normally obscure (Fig. 18), but in some cases slight projections of the cells into the ductal lumen exaggerated the intercellular borders, presenting polygonal outlines (Fig. 17 and
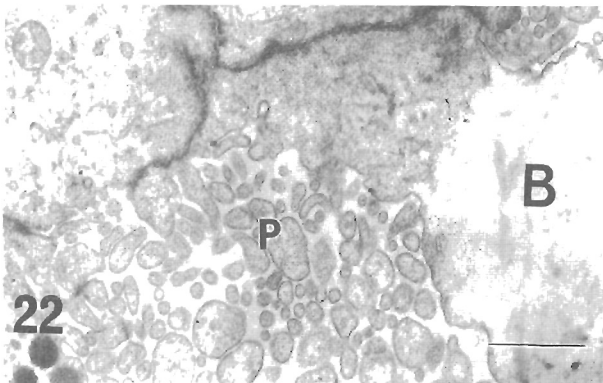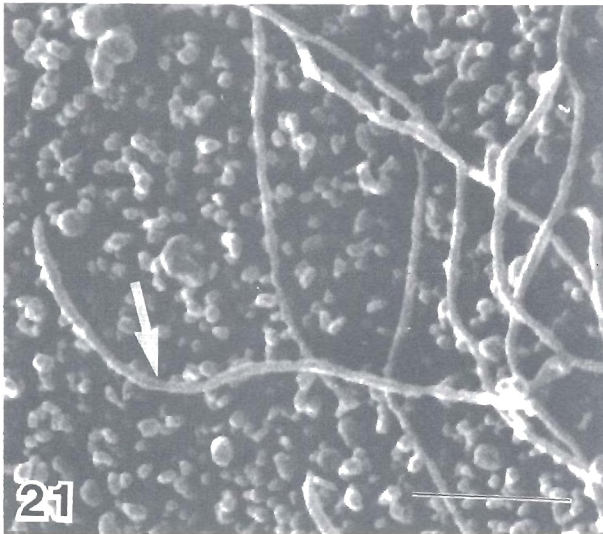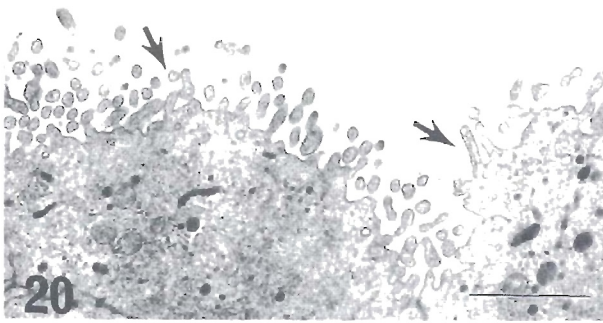
FIG. 20   A TEM micrograph of the apical region of the epididymal duct of the guinea-fowl. The microvilli (arrows) are regular in shape

Bar = 1 µm. Perfusion fixation

FIG. 21   A SEM view of the epididymal duct of the Japanese quail. The tissue was improperly fixed, and the tips of most of the microvilli are swollen

Arrow, cilium

Bar = 2 µm. Immersion fixation

FIG. 22   A TEM micrograph of the epididymal duct of improperly fixed tissue. Domestic fowl. An apical bleb (B) of a cell, and pleomorphic microvilli (P) are shown

Bar = 1 µm. Immersion fixation

19). The apical cell surface bore numerous, finger-like, uniformly distributed microvilli (Fig. 18, 19 and 20). The length of the microvilli (1,1 µm in the domestic fowl, 1,3 µm in the guinea-fowl, 1,4 µm in the turkey and 1,6 µm in the quail) was intermediate between those of the rete testis cells and those of non-ciliated cells of the efferent ducts. The apical surfaces of the Type III cells were very prone to blebbing in immersion-fixed or poorly perfused tissue (Fig. 21 and 22). When the ducts were fixed and processed with full luminal sperm content, the tips of the microvilli were usually squashed by the sperm plug, thus appearing flattened or knob-shaped. This is distinct from the pleomorphic configurations, involving the entire lengths of the microvilli of improperly fixed tissue (Fig. 21 and 22). Most of the Type III cells possessed a single, usually central cilium (Fig. 18, 19 and 21). It was not unusual to find an occasional ciliated cell, bearing a well-formed tuft of cilia, in the connecting duct of the domestic fowl.

DISCUSSION

The epididymis of the birds studied to date reveals a composite organ containing part of the rete testis, all of the efferent duct system, and connecting and epididymal ducts (Tingari 1971; Budras & Sauer 1975; Hess, Thurston & Biellier 1976; Aire 1979a; Aire, Ayeni & Olowookorun 1979). The present study has demonstrated that these ducts are lined by their respective epithelia which present similar, if not identical, characteristic features in the gallinaceous birds. They are also similar to those reported in the drake (Aire 1982a).

The surface features of the rete testis, described for the first time in this study in the Galliformes, is generally similar to that of the drake (Aire 1982b) with only a few differences being noted. The luminal surface of the rete cells in the turkey tended to possess more microvilli than those of the other species of birds, including the drake, but, generally, the presence of microvilli varied from very few and sparsely distributed to very numerous and evenly distributed. These findings are in consonance with those made in the drake (Aire 1982a) and ostrich (Aire & Soley 2000). A variant of the distribution pattern was found in the Japanese quail where the microvilli were aggregated centrally in some cells. It is not known if this disparity in number and distribution pattern of microvilli is of any functional significance. Luminal macrophages resting on the rete epithelium were numerous in the domestic fowl (present study) and in the drake (Aire 1982b), but were infrequently observed in the other species in the present study. While ultrastructural studies (TEM and SEM) have added little to our knowledge of the precise function(s) of the rete testis cell, it is, however, known that there is little net fluid transport across the rete epithelium in the Japanese quail (Clulow & Jones 1988).

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The efferent ducts constitute between 35% and 62% of the entire volume of the epididymal region of some gallinaceous birds (Aire 1979b). Both SEM and TEM findings indicate that this duct system plays an important role in fluid absorption in birds (Tingari 1972; Aire 1980; present study). Clulow & Jones (1988) have demonstrated that the efferent ducts of the Japanese quail absorb about 86% of the fluid leaving the testis. They further observed that the distal efferent duct epithelium was also actively involved in fluid re-absorption although not as much as the proximal segment, because there was a tenfold increase in the concentration of spermatozoa between the distal efferent duct and the connecting duct. However, the subapical TEM features of the non-ciliated cells of both ducts differ (Aire 1980). The proximal efferent duct may be involved in both fluid reabsorption and removal of particulate or protein material from the lumen whereas the distal segment may be involved in only fluid reabsorption. This speculation is in consonance with the organelle content in the supranuclear region of the non-ciliated (Type I) cell of the proximal efferent duct, where numerous, well-formed tubular coated pits, apical tubules, dilated vacuoles, and dense bodies abound. In the distal segment, on the other hand, these organelles are not as well developed in the Type II cells as in the Type I cells of the proximal segment, and dense bodies, probably lysosomes, are rarely observed in the supranuclear zone of the Type II cell. The highly folded mucosa of the proximal efferent duct (this study), the arrangement of the efferent duct system into a large number of narrow ducts disposed in parallel to provide a large ratio of luminal surface area to luminal volume (Clulow & Jones 1988), and the ultrastructure of the non-ciliated (Types I and II) cells (Tingari 1972; Hess & Thurston 1977; Aire 1980) are obviously consistent with fluid reabsorption activity.

The connecting duct and ductus epididymidis constitute between 2,5% and 10% of the epididymal volume of some gallinaceous birds (Aire 1979b; Clulow & Jones 1988). The SEM luminal features of the epithelial lining of these two ducts are identical, and the main cell of this epithelium is the non-ciliated (Type III) cell. The microvillous border of this cell is different from that found in either segment of the efferent duct whose microvilli were quite long and dense, and the subapical organelles do not indicate a fluid reabsorptive property for this cell. Clulow & Jones (1988) showed that little net fluid transport occurs in the connecting and epididymal ducts. A merocrine secretory role appears to be indicated for these ducts in birds (Tingari 1972; Aire 1998).

The presence of a single cilium projecting from the cell surface of the rete cells and non-ciliated (Types I, II and III) cells is intriguing. Neither Bakst (1980) nor any other previous authors have reported this phenomenon as a normal feature of the various epididymal ducts of gallinaceous birds. However, Aire (1982a, b) observed this structure in the rete testis and epididymal duct cells of the drake. He suggested that the non-ciliated (Type III) cell of the connecting ducts and epididymal duct could be termed a uni-ciliated cell. The ubiquitous nature of the single cilium in the various ducts of the avian epididymis would now cast doubt on the appropriateness and feasibility of that suggestion. The retention of the previous terminology, viz. rete cell and non-ciliated (Type I for the proximal efferent duct; Type II for the distal efferent duct, and Type III for the connecting, epididymal and deferent ducts) will prevent confusion. Nevertheless, the function of this cilium is, at best, speculative. The absence of this structure in some cells may be attributed to developmental stages of the cilium. Apparent emerging cilia have been observed in the rete testis cells in the ostrich (Aire & Soley 2000).

In this study a controlled investigation of the effect of the fixing method (immersion or vascular perfusion) on the epithelial cells of the various ducts of the epididymis was carried out with the hope that the controversy over what has been termed apocrine secretion in the avian epididymis would be advanced towards resolution. In their studies, Hess & Thurston (1977) and Bakst (1980) demonstrated numerous apical blebbings of Types I and III (their Types I and II) cells, which they were convinced represented apocrine secretory activities by these cells. Aire (1979a, 1980) and Aire, Ayeni & Olowookorun (1979) had reported that these blebs and pleomorphic microvilli were artefacts of fixation. Hess & Thurston (1977) and Bakst (1980) used the immersion (flooding) method of fixation in their studies. The present study confirms our previous conclusion that these cytoplasmic manifestations are artefactual. Perfusion fixation of the epididymal tissue of the bird does not completely eliminate apical blebbing of the non-ciliated cells in the various ducts, apparently because of time factor and oxygen deprivation, but it is largely prevented. The artefactual nature of the cytoplasmic blebbing is supported by Thurston (see Bakst 1980) who wondered why the blebs were not present in ejaculates of birds, if they were normal structures. Nicander & Glover (1973) and Hamilton (1975) regarded apical blebs, which are considered to be apocrine secretions by others (see Ilio & Hess 1994), as artefacts resulting from improper fixation.

In conclusion, the surface morphology of the excurrent ducts of the testis is quite similar in all the bird species examined in this study, and with adequate fixation, there are no artefactual apical cellular modifications which may be mistaken for apocrine secretion in any duct of the avian epididymis. The morphological features observed in these Galliform birds are also generally similar to those reported for the drake and ostrich (Aire 1982a; Aire & Soley 2000). It is quite possible that birds, in general, have similar ductal surface configurations.

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