

Ultrastructural features of the excurrent ducts of the testis of a wild bird, the cattle egret (*Bubulcus ibis*)

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ABSTRACT

The structure and functions of components of the excurrent ducts of the testis of birds are still poorly understood. Most of the few reports on these ducts are on domesticated avian species. This report on the cattle egret is one of the very few studies on wild birds. Tissues from the ducts of five sexually mature and active male birds were routinely prepared and stained for light and transmission electron microscopy. The epididymis and ductus deferens of the cattle egret are generally similar structurally to those reported for domestic birds, with a number of cellular differences. The epithelium of certain segments of the egret's rete testis displayed numerous, large intercellular spaces, not usually observed in most domestic avian species. As in the domestic species of birds, the proximal efferent ducts in the cattle egret displayed a robust endocytic apparatus as well as abundant lysosomes, but there were very few heterolysosomes and telolysosomes, which were common in domestic species. The border between the proximal and distal efferent ducts also demonstrated active spermiophagy. In addition to evidence of moderate secretory activities, the presence of extremely large heterolysosomes and telolysosomes also demonstrated the endocytic and digestive ability of cells lining the ductus deferens.

1. Introduction

The excurrent ducts of the testis of animals have always attracted and demanded attention because of their importance in the development of the male gamete, the spermatozoon, as this itinerant cell transits from the testis through to, and beyond, the epididymis (Turner, 1991; Hinton and Palladino, 1995; Janssen et al., 1998; Aire, 2007; Rinaldi et al., 2020). A considerable number of studies on the excurrent ducts of mammals indicate that each duct plays specific or complementary roles in the maturation of the spermatozoon through this journey (Axnér et al., 1999; Johnston et al., 2005; Jelinsky et al., 2007; Belleannee et al., 2012; Rinaldi et al., 2020).

The epididymis of birds is a discreet organ comprising a maze of ducts, including aspects of the rete testis, efferent ducts, connecting ducts and the epididymal duct, each within or contributing to this entity. The connecting ducts are short and link the efferent ducts to the epididymal duct, whose distal continuation is termed the ductus deferens. The connecting duct, epididymal duct and ductus deferens are lined

by a similar, if not identical, epithelium, and therefore, constitute a functional unit (Tingari, 1971, 1972; Aire, 1979a), which according to Tingari (1971, 1972) are merely different segments of the same organ, an organ equivalent to the epididymis of mammals. Aire (2007) has therefore proposed that these three component ducts, constituting about 74 % of the entire excurrent duct volume in the Japanese quail (Clulow and Jones, 1988) be collectively known as the epididymal duct unit, in birds. In birds, spermatozoa spend most of their time outside the testis in the ductus deferens (Clulow and Jones, 1988), where they develop their 'mature' pattern of motility (Clulow and Jones, 1982). Although not much is known about the specific roles of the efferent ducts, epididymal duct, and ductus deferens in birds, however, about four Wolffian duct proteins bind to spermatozoa as they pass through the epididymis and ductus deferens of the rooster, and these epididymal proteins are order-specific in birds (Esponda and Bedford, 1985; Morris et al., 1987).

In mammals, not only do the epididymal ducts vary in structure from one species to another, but also this organ is subdivided into segments (beyond the customary caput, corpus, and cauda epididymides) that

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behave as a series of organs placed side by side, with their functions varying extensively along the length of the epididymis because each segment expresses its own and overlapping genes, proteins, and signal transduction pathways (Domeniconi et al., 2016; Turner et al., 2003). The different regions of the mammalian epididymis vary tremendously between species, and hence the results from one species cannot be used to find regions for functional studies in another species of animal (Axnér et al., 1999). Besides, the principal cells along the entire epididymal duct may not appear to vary structurally, but they vary in their gene expressions and functions because Rinaldi et al. (2020) found that the multiple discrete groups of principal cells arrayed along the epididymis exhibited roughly eight unique gene expression signatures across the epididymis and vas deferens.

It is clear that similar studies in birds, based on their peculiar composition and distribution of excurrent ducts of the testis, are necessary to determine the possible pattern of gene expression and functional variations along, and within, the different ducts in the avian epididymis. The few reports on the excurrent ducts of the testis have, in the main, been on domesticated, and very few, indeed, on wild, birds (Aire, 2007). The cattle egret is a wild species of bird that has close association with various species of mammals, especially cattle, in the wild, and in organized farms as well as migrant trails. It is desirable that more studies be carried out on the male reproductive system of birds, especially of wild species, not only to contribute to knowledge of avian excurrent ducts of the testis, but also to comparative epididymology. Perhaps, more importantly, these are times when over 1300 bird species are at risk of extinction throughout the world (www.birdlife.org), and therefore captive breeding and assisted reproduction may be the only remedy to the possible extinction of these species of birds (Prieto et al., 2014; Santiago-Moreno et al., 2016). Knowledge of the reproductive systems will assist scientists with the understanding and confidence required in the use of these remedial processes and measures in birds. The purpose of this morphological study is to identify and characterise the components of the excurrent ducts of the testis in the wild, Pelicaniforme bird, the cattle egret (*Bubulcus ibis*), and to compare the ducts with those in other avian species already studied. Data from this study supplements the information on sperm structure and development in the cattle egret (Roopnarine et al., 2019; 2021), in providing a wholistic overview of the male reproductive biology of this bird.

2. Materials and methods

The birds used for this study were obtained during the dry season of Grenada (January to May), with approvals from the Ministry of Agriculture of Grenada, and the Institutional Animal Care and Use Committee (Approval #, IACUC-22010-R) of the St. George's University. The birds were captured by local trappers, using mist nets and loop-rope traps. Only birds displaying the breeding plumage of red feet and red beaks were selected, and they were euthanized by an overdose of inhalant isoflurane, or by an intracoelomic injection of pentobarbitol. The thoraco-abdominal cavity of each of the five selected birds was opened and tissues were obtained from the enlarged biologically active testes and sperm-filled ducti deferentia for the study. Fig. 1 shows the sites from which tissue for both light and electron microscopy were obtained.

For histological study, tissues were obtained from two birds and were routinely processed for light microscopy. Fixation of samples was performed by immersion in 10 % phosphate buffered formalin. Using the Leica TP1020 tissue-processor, the tissues were then immersed in the following baths: 10 % neutral buffered formalin, increasing concentrations of ethanol (70–100 %), xylene for clearing, and finally, liquid paraffin. The tissues were then embedded with the aid of a TissuePrep Embedding Compound before cutting 5 µm-thick sections in a Leica microtome RM2245. The sections, mounted on glass slides, were then processed further, and routinely stained with Haematoxylin and Eosin (H & E).

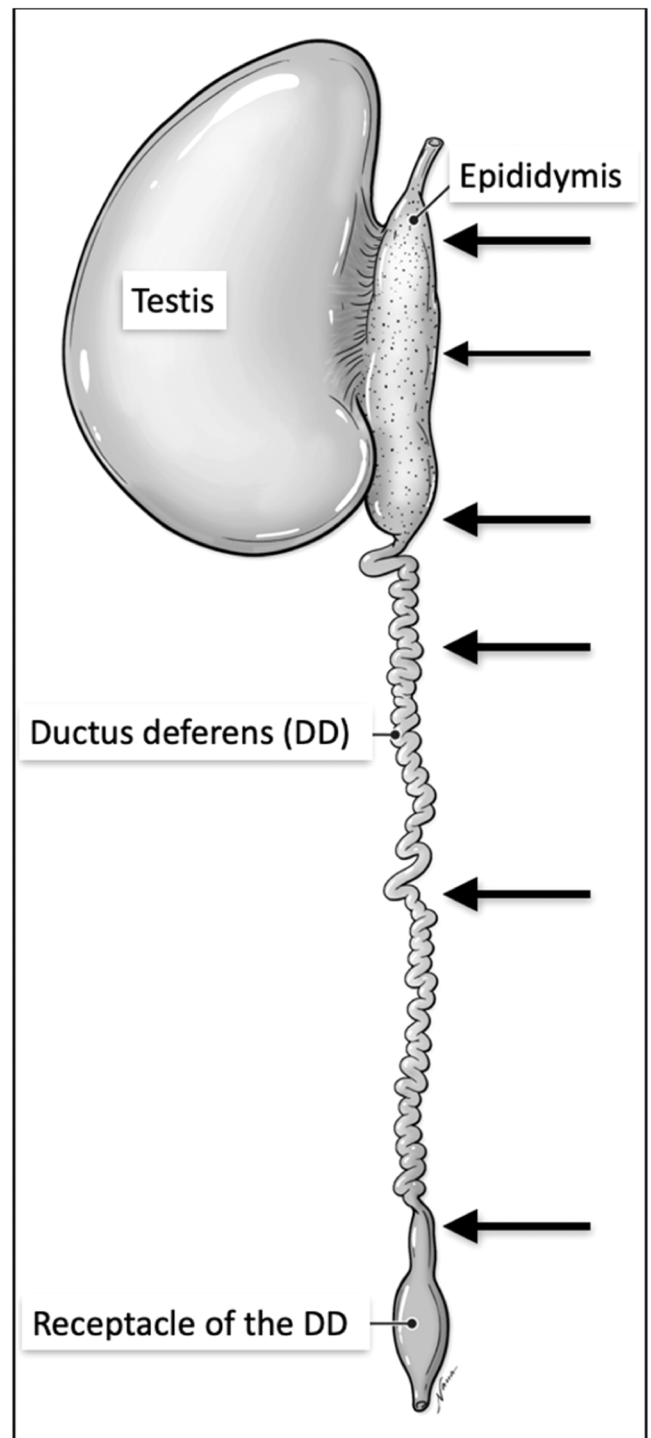


Fig. 1. A diagrammatic representation of the testis and its excurrent ducts, showing the position and relative extent of the epididymis, and ductus deferens as well as the sites of tissue harvest (arrows) for histological and ultrastructural studies. Not drawn to scale.

For transmission electron microscopy (TEM) tissues were obtained from five birds and fixed appropriately at St. George's University while processing and microscopy was performed at the University of Pretoria, South Africa. The thoraco-abdominal cavity was opened quickly, and in each of three birds, a 25-gauge catheter was inserted into the left ventricle or aorta for intravascular perfusion (Aire, 1979), using heparinized physiological saline to flush the blood out of the vessels before intravascular perfusion fixation with 3 % glutaraldehyde buffered in

0.1 M phosphate buffer, at pH 7.4. Tissues obtained from two birds (same birds used for histological study) were fixed by immersion in the same fixative (glutaraldehyde). The tissue samples from all the birds were then immersed in 2 % osmium tetroxide, buffered with 0.2 M sodium cacodylate for 1 h. Dehydration with increasing concentrations of ethanol preceded embedding in Epon 812. Survey sections, 1 µm thick, were cut and stained in toluidine blue. Ultrathin sections, 70 nm thick, were cut from appropriate areas and placed on copper grids, stained in uranyl acetate for 30 min, and lead citrate for 10 min. The sections were examined in a Phillips CM 10 transmission electron microscope operated at 80kv.

3. Results

3.1. The excurrent ducts of the testis of the cattle egret

The excurrent ducts of the testis of the cattle egret, as in other avian species previously studied, comprised a pyramidal epididymis (Figs. 1 and 2) and the ductus deferens. The epididymis comprised the rete testis, efferent duct system, and the epididymal duct system. In the cattle egret, the rete testis had an “intratesticular portion” (intratesticular rete [ITR], within the testis proper and, thus, enclosed by the testicular capsule), an “intracapsular portion” [ICR] within the testicular capsule itself, and an “extratesticular portion” within the epididymis, and designated the “epididymal rete portion” (IER) (Fig. 2).

The intratesticular rete (ITR) lacunae were found close to the testicular capsule on the medial border with the testis. The intratesticular rete (ITR) lacunae rested on the medial border of the testis, adjacent to the epididymal attachment, and passed through the testicular capsule, which formed expanded lacunae within the capsule (ICR), before entry into the epididymis (IER) (Fig. 3). The epididymal rete (IER) was located around the base of the epididymis. The rete lacunae opened into the efferent duct system, and the latter opened into the epididymal duct system. In histological sections, the various ducts were not arranged in any particular fashion, thus, sections of any duct may be found randomly within the epididymis (Fig. 2). The epididymal duct was continued distally by the long and wavy ductus deferens, whose diameter increased gradually as it extended caudally (Fig. 1). In this study, and where applicable, we adopted the nomenclature proposed by Aire et al. (1979) for the non-ciliated cells in the various ducts of the avian epididymis.

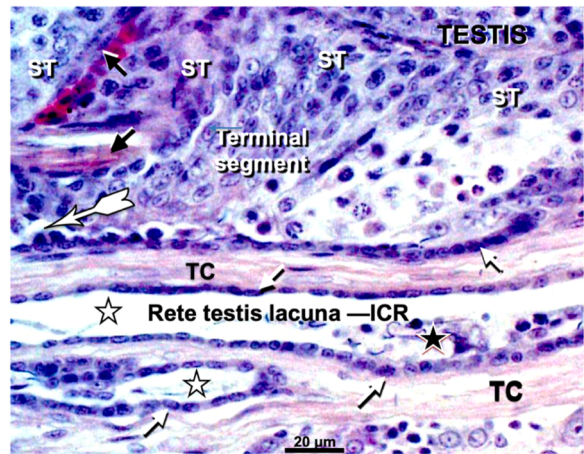


Fig. 3. A histological view of parts of the testis and its testicular capsule (TC). Black arrows = peritubular tissue of the seminiferous tubule; Arrow 12 (feathered) = lumen of the intratesticular rete testis into which opens/runs a terminal segment of the seminiferous tubule (ST); white arrows = rete testis epithelial cells; white stars = rete testis lacunae; black star = sperm and earlier germ cell series in the rete testis lumen.

3.2. General description of the composition of the epididymis of the cattle egret

The rete testis lacunae comprised three components that were found in the testis proper: 1. in the testicular capsule, 2. peripheral to the epididymis, and 3. a small testicular portion. The seminiferous tubule either ended as a terminal segment, which was continued by the intratesticular rete testis (Fig. 3) or opened directly into the rete testis. The latter continued into the testicular capsule as the intracapsular rete (Figs. 2 and 3). The lumen of the rete testis often contained a few spermatozoa and desquamated germ cells (Figs. 2 and 3). The rete testis opened into the efferent duct system made up of two segments, the proximal (PED) and distal (DED) efferent ducts. The PED generally displayed a larger duct diameter and a higher, columnar, epithelial lining than the DED (Fig. 4). In auspicious sections, the PED was seen opening into the DED, with their differing epithelial heights and cellular composition (Fig. 4). The PED epithelium was made up of the histologically lighter non-ciliated and the more eosinophilic ciliated cells, with the former being more numerous. A halo cell may be seen in the epithelium, usually in the basal aspect (Fig. 4). Whereas the luminal content of the PED had sparse content of germ cells and amorphous clumps of material, probably coagulated protein, the DED contained a compact mass of spermatozoa (Fig. 4). The DED opened into a relatively short connecting duct, which, in turn, opened into the epididymal duct, with both ducts constituting the first two components of the epididymal duct unit, and were lined by a similar, if not identical, epithelium (Fig. 4). As with the DED, the lumina of the connecting and epididymal ducts contained clumps of spermatozoa in histological sections. The epididymal duct was continued distally by a long and wavy ductus deferens, the latter acting as the main storage site for sperm in the avian reproductive tract (Fig. 4).

3.3. Ultrastructure of the duct systems of the epididymis

3.3.1. Rete testis

Ultrastructurally, the epithelium of the rete testis was squamous to cuboidal. In some sections, portions of epithelial cells were seen overlying adjacent cells (Fig. 5). The luminal surfaces of the epithelial cells projected a few stubby, microvilli into the duct lumen, and large intercellular spaces were observed between adjacent cells (Figs. 5 and 7). Mitochondria were scattered within the cytoplasm but mostly found in the supra and para-nuclear regions of the cells. Occasionally, a

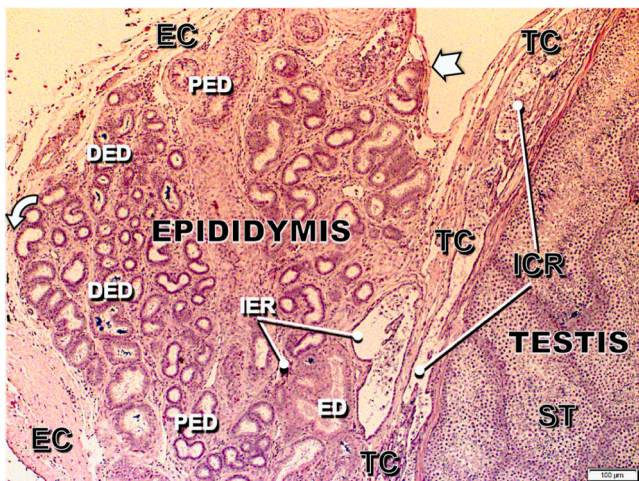


Fig. 2. This is a low power panoramic view of the epididymis, attaching to the testis by its base (notched block arrow); curved arrow = apex of the epididymis; ST = seminiferous tubule; ICR = intracapsular rete; TC = testicular capsule; EC = epididymal capsule; IER = intra-epididymal rete; PED = proximal efferent duct; DED = distal efferent duct; ED = epididymal duct.

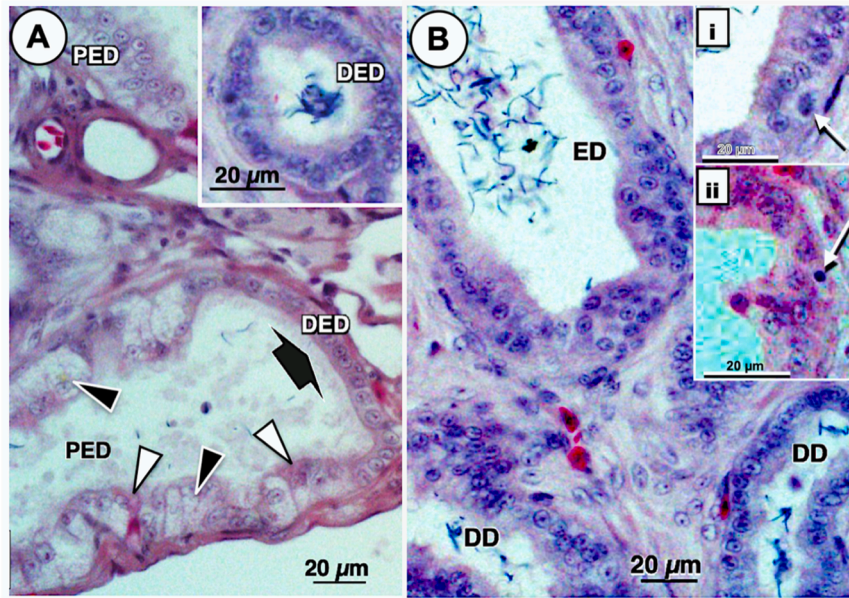


Fig. 4. Some components of the excurrent ducts of the testis in histological sections of the epididymis. In sub-figure A, a longitudinal section of the proximal efferent duct (PED) seen opening (black broad arrow) into the distal efferent duct (DED); black arrowheads = non-ciliated cells and white arrowheads = ciliated cell of the PED. Sub-Fig. B shows histological sections of the distal efferent duct (DED), the epididymal duct (ED), and ductus deferens (DD); Sub-figures B, i and ii show halo cells (white arrows).

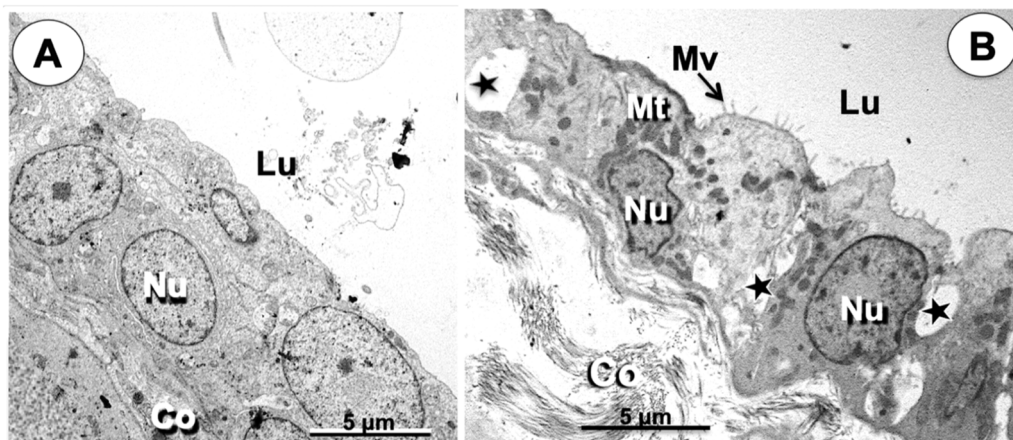


Fig. 5. Ultrastructure of parts of the epithelial lining of the rete testis lacunae. In sub-figure A, squamous epithelial lining enclosing the duct lumen (Lu); Nu = nucleus; sub-epithelial collagen = Co. In sub-figure B, epithelial cells project microvilli (Mv) into the duct lumen; black stars = enlarged intercellular spaces; Mt = mitochondria.

cytoplasmic slip from an adjacent cell, containing numerous vacuoles (Fig. 6), overlay part of an adjacent cell displaying a more electron-lucent cytoplasm. The slip of cytoplasm was filled with vacuoles that were generally empty but sometimes displayed homogeneously flocculent material (Fig. 6).

A magnification of the intercellular spaces (Fig. 7) displayed an amorphous content, and the basal part of the cell displayed a complex intertwining of the cellular membrane (Fig. 7B).

3.3.2. Proximal efferent duct

A survey image of the columnar epithelial lining of the proximal efferent duct (PED) comprised both non-ciliated (NC) and ciliated (CC) cells, with the former being more numerous (Fig. 8). The surface lining of the NC cells was thrown into a lush, closely-packed long microvilli. At low magnification, the subapical cytoplasm of the cell, was found to be devoid of organelles or obvious structural features (Fig. 9). The supra-

nuclear region was replete with dense bodies and mitochondria, but a few were present in the subnuclear zone of the cytoplasm (Fig. 9). The nuclei were generally oval and centrally located in the cytoplasm. The CC cells often appeared truncated, with well-formed cilia projecting into the duct lumen, and the nuclei were more apically located than in the NC cell. A low magnification, panoramic view of the epithelial lining of the PED showed that the sub-apical region of the NC cell appeared to be clear of organelles or structural features (Figs. 8 and 9). Below this region, in the supra-nuclear area, the cytoplasm was rich in dense, globular bodies of variable electron-density interspersed with smaller mitochondria (Figs. 8, 9 and 11). The peritubular tissue was made up of fibroblasts, smooth muscle cells and collagen bundles (Figs. 9 and 12).

At higher magnifications, the sub-apical cytoplasm formed a thick layer of an elaborate endocytic system, comprising tubular coated pits, apical tubules, large, coated vesicles or endosomes, and lysosomes (Fig. 10). Several small coated and uncoated vesicles also occurred

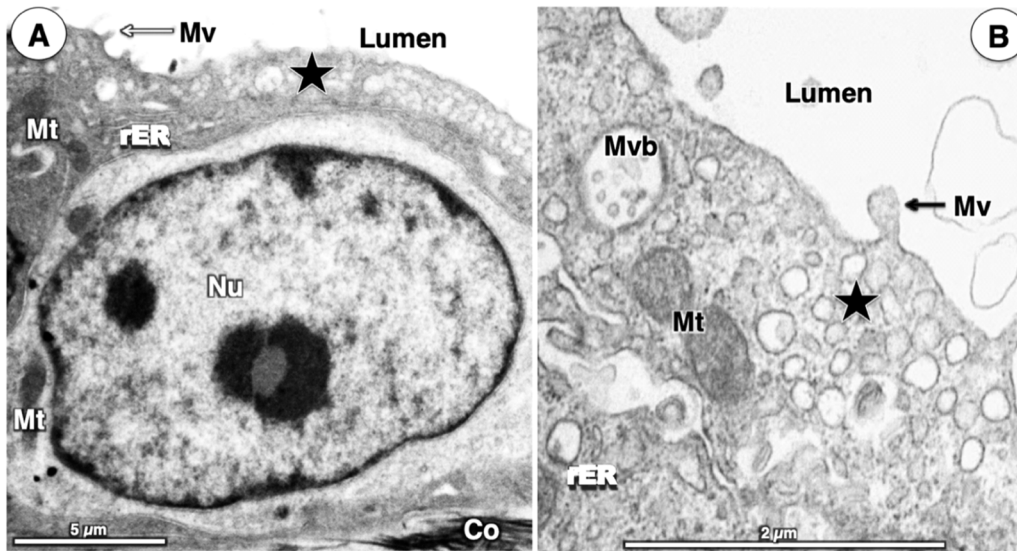


Fig. 6. The rete testis epithelial cells. In sub-figure A, a cytoplasmic slip (black star) of a rete testis epithelial cell overlying an adjacent cell with an electron-lucent cytoplasm, contains numerous electron lucent vacuoles. Mv = microvilli; Mt = mitochondria; rER = rough endoplasmic reticulum; Nu = nucleus; Co = collagen bundles. In sub-figure B, Mvb = multi-vesicular body; black star = numerous electron-lucent vacuoles in a slip of cytoplasm overlying an adjacent cell.

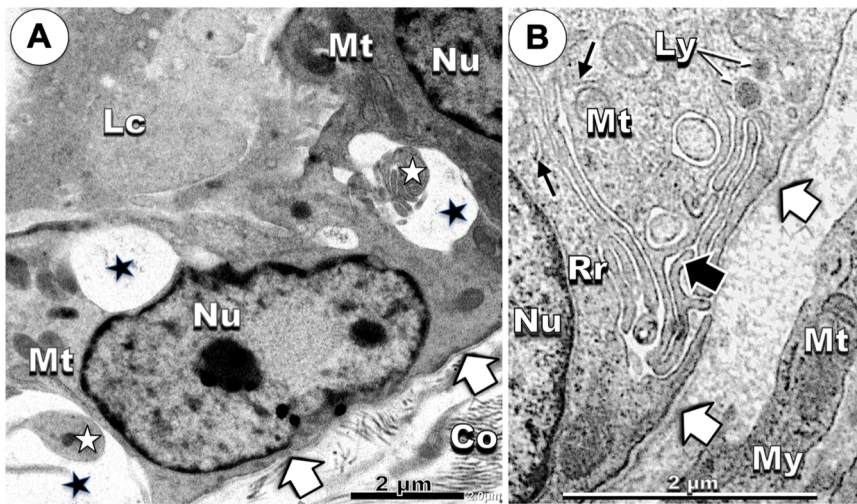


Fig. 7. A higher power view of some parts of the epithelial lining of the rete testis. In sub-figure A, the lumen of the duct contains amorphous debris (Lc); Mt = mitochondria; black star = enlarged intercellular spaces; white star = microvilli and debris in the intercellular space; Nu = nucleus; white block arrows = basal lamina. Sub-Fig. B shows the basal part of the cell. Black block arrow = intertwined cell membranes; white block arrows = basal lamina; Rr = free ribosomes; Ly = lysosomes; black thin arrow = rER. My = peritubular myoid cell.

among these members of the endocytic system. Very few or no mitochondria were seen in this zone, but a few rER profiles intermingle with the endocytic system.

The subnuclear region of the cytoplasm also displayed numerous dense bodies, but not as numerous as in the supra-nuclear region (Fig. 11). Mitochondria were also abundant in this zone of the cytoplasm and were usually closely associated with profiles of rER that tend to surround them, while some mitochondria were very closely associated with, or attached to, contiguous desmosomes (Fig. 11). These non-ciliated cells were similar in most particulars to the non-ciliated type I cells of galliform birds (Aire et al., 1979, Aire, 2007), and therefore we named it the 'NC type I cell' of the egret's excurrent duct system.

The ciliated cells were more electron-lucent than the non-ciliated cells. Their cilia extended prominently into the duct lumen and the basal bodies occupied the immediate sub-apical cytoplasm. Numerous mitochondria, smaller than those of the non-ciliated cells, occurred in

the supranuclear region of the cytoplasm (Fig. 12). The nuclei were euchromatic and irregular in outline and usually located in the luminal half of the cell. Only a few mitochondria occurred in the subnuclear region where prominent, thin and elongated RER were scattered within the cytoplasm.

3.3.3. Distal efferent duct

The distal efferent duct epithelium appeared cuboidal to columnar and comprised non-ciliated (NC) and ciliated (CC) cells, with the latter being more numerous (Figs. 13 and 14). The microvillous brush border of the apical surface of the NC cells comprised regular, moderately long, microvilli, and a solitary cilium was seen in fortuitous or favourable sections, while the CC cells projected relatively long cilia into the lumen. The nuclei of the NC cells were large, euchromatic, linearly elongated along the long axes of the cells, and occupied the basal half of the cytoplasm. The sub-apical cytoplasm had very sparse organelle content,

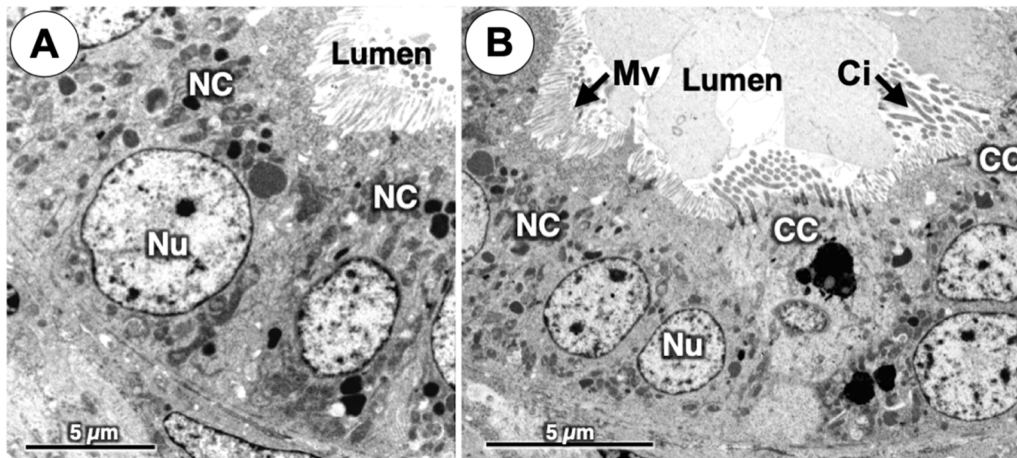


Fig. 8. Panoramic views of the epithelial lining of the proximal efferent duct (PED). In sub-Figs. A and B, the non-ciliated (NC, type I) cells alternate with ciliated (CC) cells; Nu = nucleus. In sub-figure B, NC = non-ciliated cell; CC = ciliated cell; Nu = nucleus; Ci = cilia; Mv = microvilli.

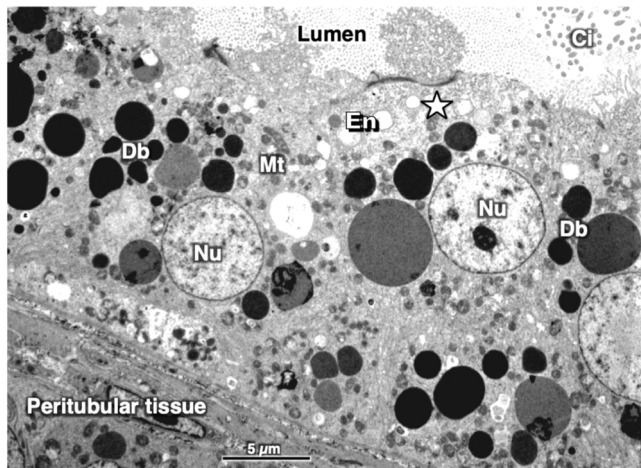


Fig. 9. A medium power view of a typical epithelium of the PED, showing cilia (Ci) of ciliated cells; white star = electron-lucent zone of the sub-apical cytoplasm of non-ciliated cells; Db = dense bodies in the supranuclear, para-nuclear and subnuclear regions of the non-ciliated cells; En = endocytic vacuoles; Mt = mitochondria; Nu = nuclei.

save for a few, small, oval-shaped mitochondria (Fig. 13), while numerous mitochondria were scattered throughout the peri-nuclear and subnuclear regions of the cytoplasm (Figs. 13 and 14).

The epithelium occasionally displayed an intraepithelial lymphocyte between the epithelial cells (Fig. 14). The sub-apical cytoplasm displayed profiles of sparsely granulated or smooth surfaced endoplasmic reticulum (Fig. 14). The supra-nuclear region showed most of the cell's mitochondria. Several elongated, thin profiles of rER were scattered throughout the cytoplasm (Figs. 14 and 15). The non-ciliated cell in the DED was, again, similar in most particulars to the non-ciliated type II cell of galliform birds (Aire et al. 1979; Aire, 2007), and therefore similarly and appropriately classified, as such, for the egret.

The ciliated cells (CC) had a more electron-lucent cytoplasm than the NC cells (Figs. 14, 15, and 16), and the subapical region contained the basal bodies of the flagella and a few, relatively smaller mitochondria in comparison to the non-ciliated cells. The nuclei were linearly arranged, euchromatic, irregular in profile, and situated in the mid-region or occasionally in the basal part of the cell. Most of the mitochondria were situated in the supranuclear region, with fewer in the para- and subnuclear regions. There were more numerous, scattered, long profiles of rER in the supranuclear region, and fewer in the subnuclear region of the

cytoplasm. A few mitochondrial-desmosome complexes (DMCs) were also seen between some adjacent cells, irrespective of type of cell, non-ciliated or ciliated.

At the junction between the PED and DED, spermiophagy was observed with numerous sections of spermatozoa within phagocytic vacuoles in the non-ciliated cells (Fig. 16). As in the proximal efferent duct, a few lymphocytes occurred in the epithelium.

3.4. The epididymal duct system (connecting duct, epididymal duct, and ductus deferens)

3.4.1. The connecting and epididymal ducts

The connecting duct, epididymal duct, and ductus deferens displayed a similar epithelial lining (Figs. 17 and 18), and will be described together. The connecting ducts and the ductus deferens had a shorter epithelium than the epididymal duct. Panoramic views of connecting ducts showed narrower, round or oval transverse sections than the epididymal duct (Fig. 17), but both ducts contained plugs of spermatozoa. An occasional ciliated cell was seen among the non-ciliated cells that constitute the epithelium of this epididymal duct system (Fig. 17). The apical surface displayed numerous, moderately long, microvilli which projected into the duct lumen perpendicularly (Figs. 17 and 18). The relatively euchromatic nuclei were round or oval in shape and located in the mid to basal part of the cell (Fig. 17). The nuclei were generally surrounded by mitochondria on all sides, although more numerous in the supra-nuclear region of the cytoplasm. The epithelial lining of the epididymal duct was higher than in the connecting duct and ductus deferens (Fig. 18). The nuclei were linearly elongated, pyramidal in shape, and occupied the basal two-thirds of the cytoplasm. Two nucleoli were commonly observed in the nucleoplasm. A few lipid droplets lied at the proximal and/or distal poles of the nuclei.

The microvillous brush border comprised numerous, regular, moderately elongated microvilli, which were sometimes branched (Figs. 17, 18 and 19). The sub-apical cytoplasm contained a few small, coated vesicles, numerous transverse sections or elongated profiles of granulated and sparsely granulated reticulum, with the latter being more numerous (Fig. 18). The mitochondria were large and appeared in various shapes but mostly elongated. The subnuclear cytoplasm showed a few scattered mitochondria with interspersed elongated profiles of rough endoplasmic reticulum (rER). One or two dense bodies or lysosomes might be seen close to the anterior or posterior pole of the nucleus (Figs. 17 and 20). The basal aspect of the epithelial cell did not exhibit any striking organelle or structural configuration, save for a few mitochondria and thin, elongated profiles of rER (Fig. 20). An occasional dense lysosome was seen below the nucleus. Basal cells, lying on the

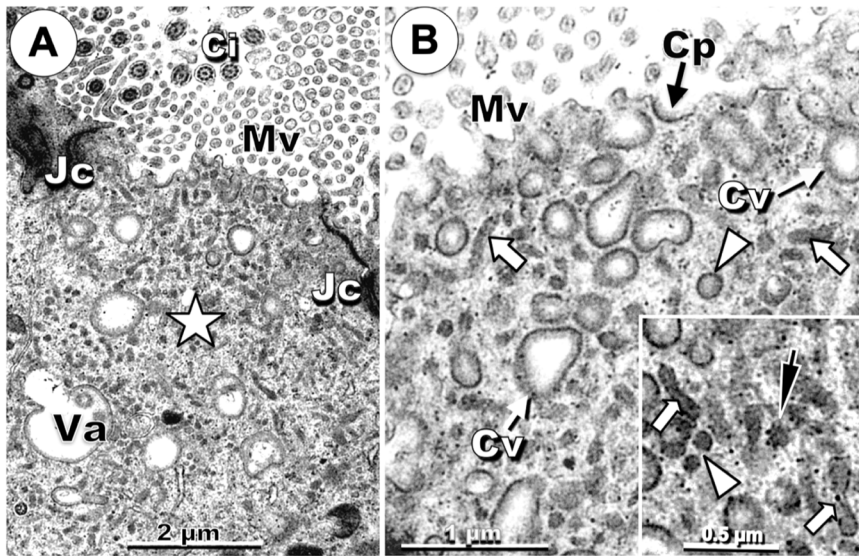


Fig. 10. Sub-Fig. A shows a low power view of the sub-apical region of the cytoplasm of the non-ciliated cell of the PED; Ci = cilia; Mv = microvilli; Jc = junctional complex; white star = subapical region of the cytoplasm displaying silhouettes of the endocytic system of the NC type I cell; Va = endocytic vacuole. Sub-Fig. B is a higher power view of the endocytic system in the subapical region of the cell. Cp = coated pit; Cv = large coated vesicles; white block arrows = longitudinal profiles of apical tubules; white arrowheads = transverse sections of apical tubules. The inset shows longitudinal profiles of sparsely coated or smooth endoplasmic reticulum (white block arrows), black arrow = bristle coated vesicle; white arrowheads = transverse sections of apical tubules.

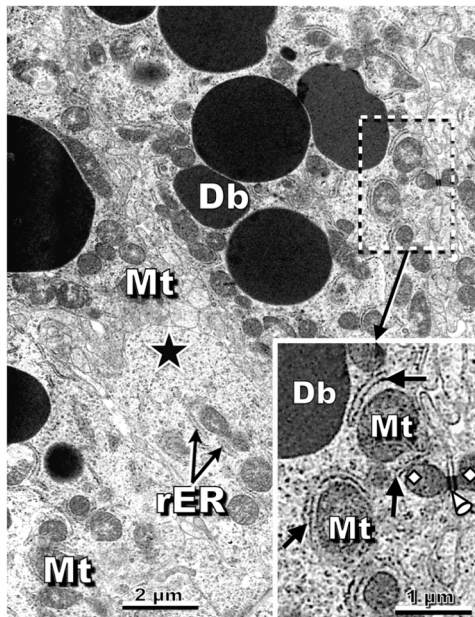


Fig. 11. Higher power view of the basal cytoplasm of the non-ciliated (type I) cells. There are numerous scattered mitochondria (Mt), variably-sized dense bodies (Db), few, scattered profiles of rough endoplasmic reticulum (rER); black star = numerous free and rosettes of ribosomes. In the inset, which is an enlargement of the marked area (broken-line rectangle) in the main figure; Mt = mitochondria closely surrounded by profiles of rough endoplasmic reticulum (black arrows), forming the 'endoplasmic reticulum-mitochondria encounter structure' (ERMES); Db = dense body; white diamond = mitochondria associated with a desmosome (cone 2), forming the 'desmosomal-mitochondrial complexes' (DMC).

basal lamina, were seen in the epithelium of the epididymal duct unit (Fig. 21). The number increased caudally along the epididymal duct. The moderately euchromatic nuclei of basal cells were usually horizontally elongated and surrounded by clumps of microfibrils. Only a few organelles occurred in the cytoplasm. Mononuclear immunological cells

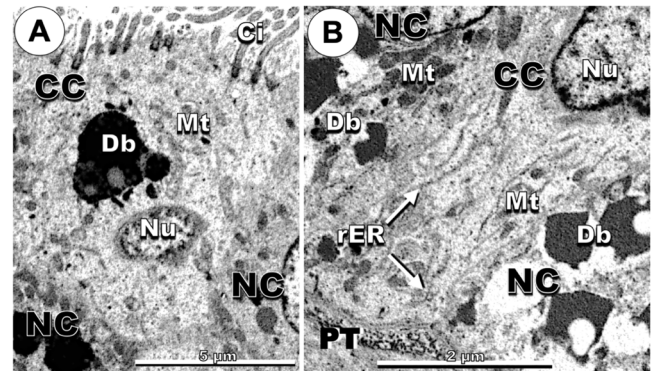


Fig. 12. Ciliated cells of the PED. Sub-Fig. A shows the supranuclear region of the cytoplasm, with numerous mitochondria (Mt); Ci = cilia; Nu = irregularly shaped nucleus. Sub-Fig. B shows the basal parts of non-ciliated (NC) and ciliated (CC) cells. The latter display numerous, thin, profiles of rough endoplasmic reticulum (rER). Db = dense bodies in adjacent non-ciliated cells. PT = peritubular tissue.

also occurred as described for the earlier duct series (Fig. 21). The nuclei may be irregular in outline and were heterochromatic. The cytoplasm was electron-lucent, and the organelle content was sparse. These cells occurred at any level within the duct epithelium.

3.4.2. The ductus deferens

The epithelial lining of the ductus deferens was mostly cuboidal, but comprised non-ciliated cells, as found in the connecting and epididymal ducts (Fig. 22); an occasional ciliated cell appeared in the epithelium. The cytoplasm was moderately electron-dense. The nuclei were oval or elongated horizontally within the cell and often occupied most of the cytoplasm of the cell (Figs. 22 and 23). Since the duct was usually convoluted, profiles of the duct epithelium were found lying adjacent to each other, separated by peritubular tissue of myoid cells and bundles of collagen (Figs. 22 and 23). One or two large dense bodies (Db) occupied a relatively large proportion of the cytoplasm (Figs. 22 and 24). The nucleus was surrounded by mitochondria and profiles of elongated rER

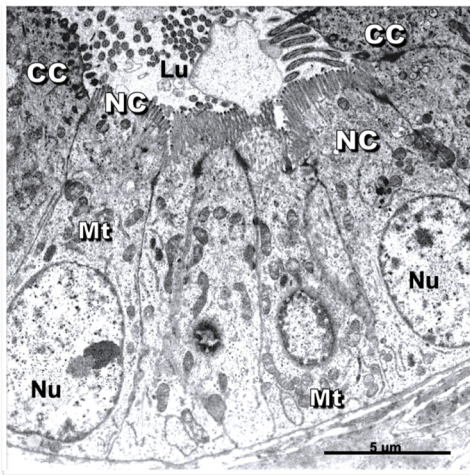


Fig. 13. A panoramic view of the epithelium of the distal efferent duct (DED). Lu = lumen of the duct; CC = ciliated cell; NC = non-ciliated type II cell; Mt = mitochondria; Nu = nucleus.

(Fig. 23). In favourable sections of the epithelium of the ductus deferens, a primary cilium (also known as sensory or solitary cilium) was seen projecting, along with the microvilli of the epithelial cells, into the duct lumen (Fig. 24). The microvillous brush border was not as luxuriant as found in the efferent duct system. The cellular membranes of adjacent cells intertwined very complexly and elaborately (Figs. 24, 25, and 26). When present, the dense bodies, regarded as heterolysosomes, were very large, and observed to displace the nucleus or indent it (Figs. 22 and 24). The sub-apical cytoplasm was generally free of organelles, but a few small mitochondria were present, along with small multi-vesicular bodies (Mvb) and a few autophagosomes (Fig. 25). At higher magnification, the most obvious organelles in the sub-apical cytoplasm were

transverse sections of sparsely coated ER, but a few small, coated vesicles also occurred (Fig. 25; Inset). The Golgi complex was small, relative to that of the epididymal duct. The basal part of the cell displayed mitochondria surrounded by well-formed strands of rER, and a remarkable array of contiguous mitochondria were seen attaching to desmosomes (Fig. 26). As was the case in the efferent duct system, the non-ciliated (type III) cell in the epididymal duct system of the cattle egret was similar structurally in all its component ducts, but dissimilar to the non-ciliated types I and II cells in the efferent ducts, and therefore we have adopted the 'NC type III cell', employing the nomenclature of Aire et al. (1979a), for the homologous cell in the egret excurrent duct system.

4. Discussion

4.1. General

For the purpose of appropriateness and avoidance of confusion, the cell types in the avian epididymis have been classified as 'rete cell', in the rete testis and 'non-ciliated cells' [Type I in the proximal efferent duct, Type II in the distal efferent duct, and Type III in the connecting, epididymal and deferent ducts (Aire et al., 1979a; Aire, 2000a; Aire and Josling, 2000; Aire, 2007). Fig. 27 shows the differences between the three cell types.

The epididymis of the cattle egret is similar in structure and position to that of some members of the Galloanserae (Aire, 2007), but it is very small, apparently on a relative basis. The ductal composition is also similar to that described for the domestic birds, mainly members of the Galloanserae (Tingari, 1971; Hess and Thurston, 1977; Budras and Sauer, 1975; Aire, 1979a; Aire, 2007; Aire et al., 1979).

The component epithelial cells in the various duct units in the egret are similar, in most particulars, to those in other birds studied (see Aire, 2007), thus the nomenclature was applied to the corresponding cells of the egret.

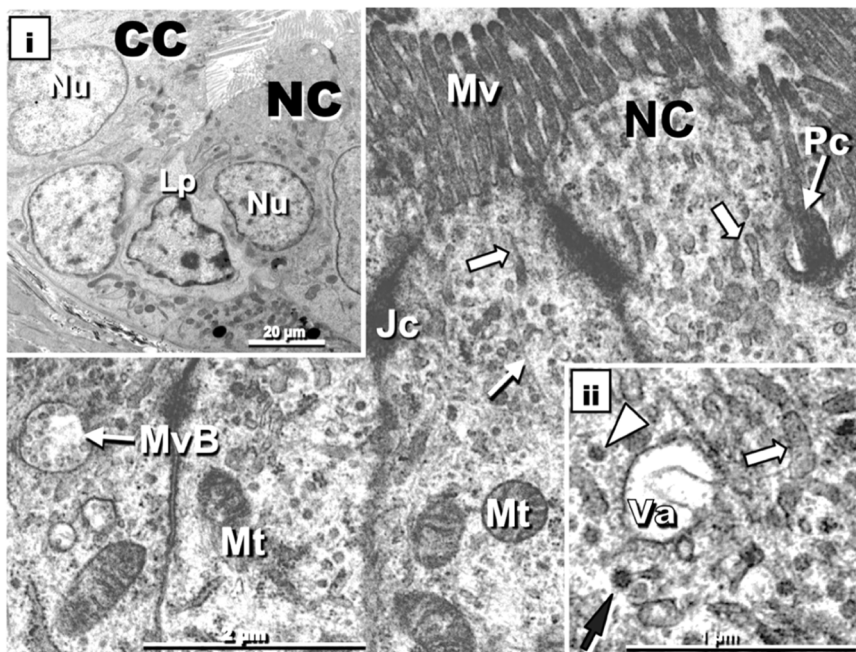


Fig. 14. The inset i figure shows a low power view of the epithelial lining of the DED, displaying ciliated (CC) and non-ciliated (NC) cells; Nu = nucleus; Lp = intraepithelial lymphocyte or halo cell. The main figure shows the supranuclear segments of the non-ciliated (NC II) cells: Mv = microvilli; Pc = primary cilium or solitary cilium. The supranuclear regions of the cells show an electron-lucent subapical zone of the cytoplasm comprising mainly elongated profiles of sparsely granulated or smooth endoplasmic reticulum (block arrows). A few multivesicular bodies (MvB) may be seen among these tubules. Distal to this zone are large, scattered mitochondria (Mt) and a moderate number of rough endoplasmic reticulum (rER); white arrow = microtubule. Inset ii shows higher power view of the sub-apical smooth or sparsely granulated endoplasmic reticulum (block arrow), small dense vesicles (arrowhead), bristle-coated vesicles (black arrow) and a vacuole (Va).

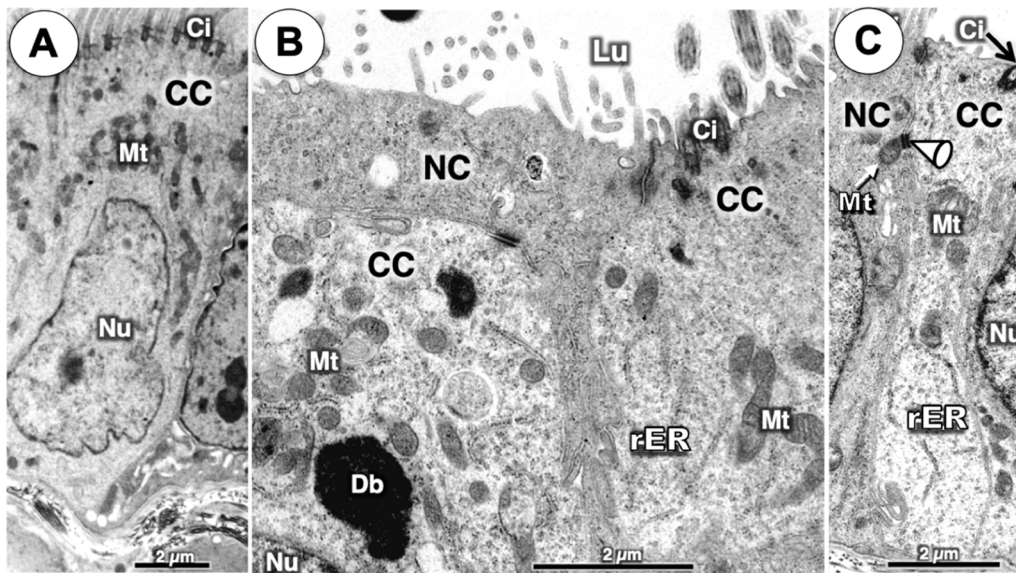


Fig. 15. The DED epithelial lining showing mostly ciliated cells (CC), with a few adjoining non-ciliated cells (NC). Sub-Fig. A is a low power view of the ciliated cell (CC); Ci = cilia; Nu = elongated, irregularly-shaped nucleus; Mt = mitochondria. In sub-figure B, a slip of electron-dense cytoplasm of a NC cell overlies and runs between two CC cells. Lu = lumen; rER = rough endoplasmic reticulum; Db = dense body. In sub-figure C, the para-nuclear aspect of the CC cell shows sparse organelles; cone 2 = desmosome associated with mitochondria.

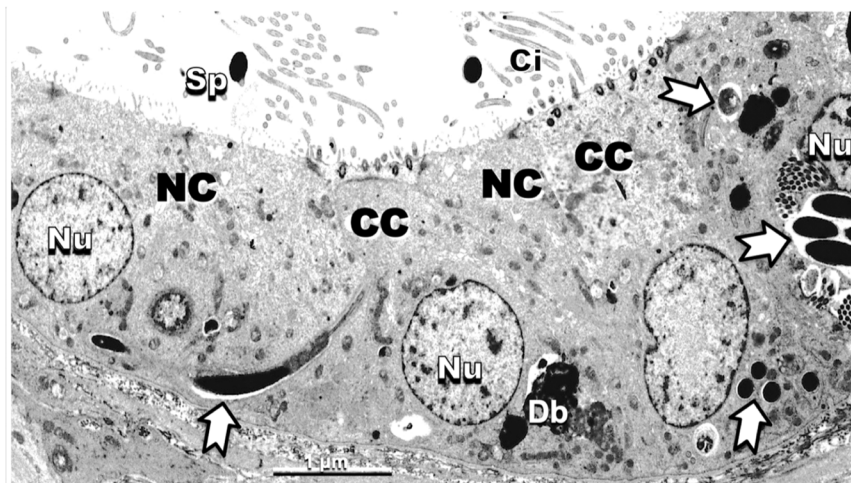


Fig. 16. A junctional area between the PED and DED, showing avid spermioophagy in the non-ciliated (NC = type II) cells, but not in the ciliated (CC) cells. Sp = spermatozoon in the duct lumen; Ci = cilia; notched arrow = sections of spermatozoa in phagocytic vacuoles; Db = dense body; Nu = nucleus.

4.2. The rete testis unit

The rete testis epithelium and component cells are generally similar to those of birds already studied (Tingari, 1971, 1972; Hess and Thurston, 1977; Osman, 1980; Budras and Meier, 1981; Aire, 1982a; Aire, 1982b; Stefanini et al., 1999; Aire and Soley, 2002). However, in the egret, some epithelial linings are slightly different from others in the structure of certain component epithelial cells. For example, a few rete cells are more electron-dense than others and in some epithelia; such cells have relatively large intercellular spaces, which contain foci of amorphous material comprising dense content. This structural feature is indicative of an epithelium that absorbs considerable amount of fluid from the duct lumen (Pudney and Fawcett, 1984), but Clulow and Jones (1988) have shown, in the Japanese quail, that there is little net fluid transport through the rete testis epithelium. The significance of these baso-lateral dilations between certain rete testis cells in the egret is not clearly understood. It is also not understood why strips/slots of

cytoplasm overlying adjacent neighbouring cells, in tangential sections, contained an abundance of vacuoles. This structural feature has also been observed in the drake (Aire, 1982) and in the Italian variety of the quail (Orsi et al., 2009).

4.3. The efferent duct unit

The proximal (PED) and distal (DED) efferent ducts of the cattle egret are also generally similar to those of other birds (Tingari, 1971; Budras and Sauer, 1975a; Hess et al., 1976; Hess and Thurston, 1977; Aire, 1979a, 1980; Aire et al., 1979; Budras and Meier, 1981; Bellamy and Kendall, 1985), as well as mammals studied (Reid and Cleland, 1957; Ilio and Hess, 1994), in which the duct linings display two epithelial cell types, ciliated and non-ciliated (Reid and Cleland, 1957; Ilio and Hess, 1994). In the egret, the endocytic apparatus of the PED non-ciliated cell, classified as non-ciliated cell type 1 (NC 1) (Aire et al., 1979; Aire, 2007), is very well developed, and the dense bodies, regarded as

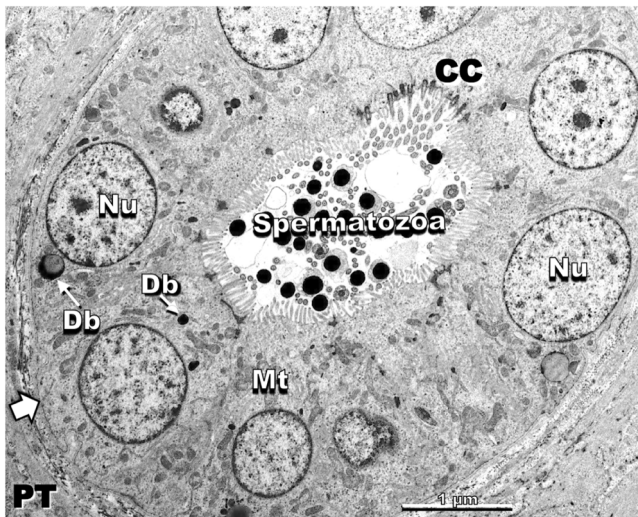


Fig. 17. A low power view of a transverse section of the connecting duct. An occasional ciliated cell (CC) occurs in the epithelium which is lined normally by the NC type III cells. Mt = mitochondrion; Nu = nucleus; Db = dense body; white block arrow = basal lamina; PT = peritubular tissue.

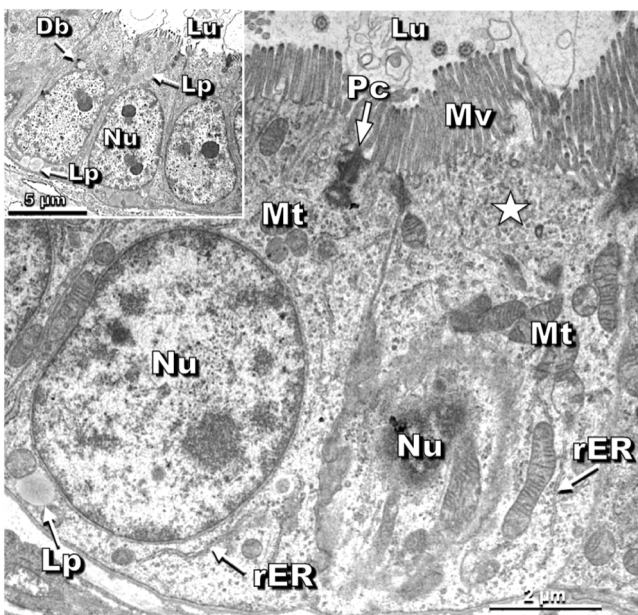


Fig. 18. The inset is a panoramic view of the cuboidal to low columnar epithelial lining of the ductus deferens, displaying elongated nuclei (Nu) occupying two-thirds of the cytoplasm; Lp = lipid droplets; Db = dense body; Lu duct lumen. The main figure displays a primary/solitary cilium (Pc), Mv = microvilli that are shorter than those of the efferent ducts, Mt = mitochondria, Nu = nucleus; white star = electron-lucent sub-apical region of the cell (NC III), rER = rough endoplasmic reticulum, Lp = lipid droplet.

lysosomes, are mostly homogenous (i.e., primary lysosomes), unlike in the galliform birds in which there are large numbers of heterolysosomes and telolysosomes occurring commonly in the cytoplasm (Aire, 2007). It is either that the NC 1 cells of the egret birds studied did not take up much of intraluminal material that required digestion or that the cattle egret NC I cells were capable of eliminating most of the heterolysosomes as quickly and efficiently as possible before the commencement of the sexually active phase of the reproductive cycle. However, the PED of the egret is structurally endowed with the appropriate cytological apparatus for absorption and degradation of material from the duct lumen, as in

most birds studied (Aire, 1979a; Aire et al., 1979; Aire, 1980; Ozegbe et al., 2006), including the Japanese quail (Aire, 1979a) in which 86 % of the fluid leaving the testis is reabsorbed there, and only 6.5 % in the DED (Clulow and Jones, 1988). About 93 % of the protein entering the ductuli efferentes of the quail is reabsorbed by these ductules (Clulow and Jones, 2004). In mammals, the non-ciliated cells have been found to interiorise material from the duct lumen by means of both fluid phase and adsorptive endocytosis (Hamilton et al., 1977; Hermo and Morales, 1984). These cells reabsorb over 90 % of the water and some ions (Clulow et al. 1994, 1998; Hansen et al., 2004) as well as some proteins (Veeramachaneni et al., 1990) before the through-flow and its spermatozoa enter the epididymis. Hess (2002) observed that water reabsorption by the efferent ducts is as efficient as in the kidney.

Macrophages have been observed in the lumen of the excurrent ducts of the testis of certain birds, particularly in the rete testis (Nakai et al., 1989; Aire, 1982a; Aire and Josling, 2000), but not in the cattle egret (this study), nor did Barker and Kendall (1984) identify macrophages in the rete testis of certain wild birds that they studied. The significance of this disparity between certain domestic species of birds and these studies in a few wild species of birds is not clearly understood. Many macrophages in the lumen of the rete testis actively phagocytized spermatozoa in the domestic fowl (Nakai et al., 1989). Epithelial cells of the rete testis had sparse coated vesicles and did not show spermio-phagy. However, the border area between the proximal and distal efferent ducts in the egret displayed avid spermio-phagy. In vasectomized quail, considerable ingestion of spermatozoa was revealed in the non-ciliated (Type I) cells of the epithelial lining of the initial part of the proximal efferent duct, at and a little beyond its junction with the rete testis (Aire, 2000a). On the other hand, spermio-phagy was observed consistently and mainly in the epithelial lining of the seminal glomus and ejaculatory duct in each of three passerine species (Chiba et al., 2011). The conditions that instigate spermio-phagy in normal animals are unknown, but it is probably a means of removing worn-out or damaged spermatozoa and other germ cell series in the seminal flow.

4.4. The epididymal duct unit

The connecting duct, epididymal duct, and ductus deferens, together, constitute the epididymal duct unit (Aire, 2007) because their epithelial linings are similar structurally and functionally. The egret epididymal duct unit is also generally similar, in design and epithelial cell composition, to that described in other species of birds (Lake, 1957; Tingari, 1971, 1972; Budras and Sauer, 1975a; Budras and Meier, 1981; Aire, 1979a; Aire et al., 1979; and Aire, 2000a). This unit comprises an epithelial lining that is constituted mainly by the non-ciliated type III epithelial cell (Aire, 2007). The brush border in all segments of the duct unit bears relatively short and regular microvilli. The presence of the non-motile primary cilium (also known as solitary cilium) in the efferent ducts and epididymal duct system of the cattle egret is in accord with similar observations in the duck (Aire, 1982b), certain gallinaceous birds (Aire and Josling, 2000), and the ostrich (Aire and Soley, 2000). It is tempting to think that this organelle is probably common to most, if not all, members of the Class of birds. The primary cilium has been described in several vertebrates, and according to Hildebrandt et al. (2011), this phenomenon appears to have a high degree of evolutionary conservation. There is already evidence that primary cilia are potent signalling organelles or sensors which are able to respond to environmental cues in the extracellular microenvironment following mechanical or chemical stimulation (Singla and Reiter, 2006; Satir et al., 2010; Fry et al., 2014; Spasic and Jacobs, 2017; Girardet et al., 2019). The dysfunction of primary cilia is now known to be associated with impaired male reproductive tract development and fertility (Girardet et al., 2019).

The intercellular membranes are complexly and intricately folded especially in the ductus deferens. This phenomenon probably stabilizes intercellular attachments and, thus, the structural integrity of the

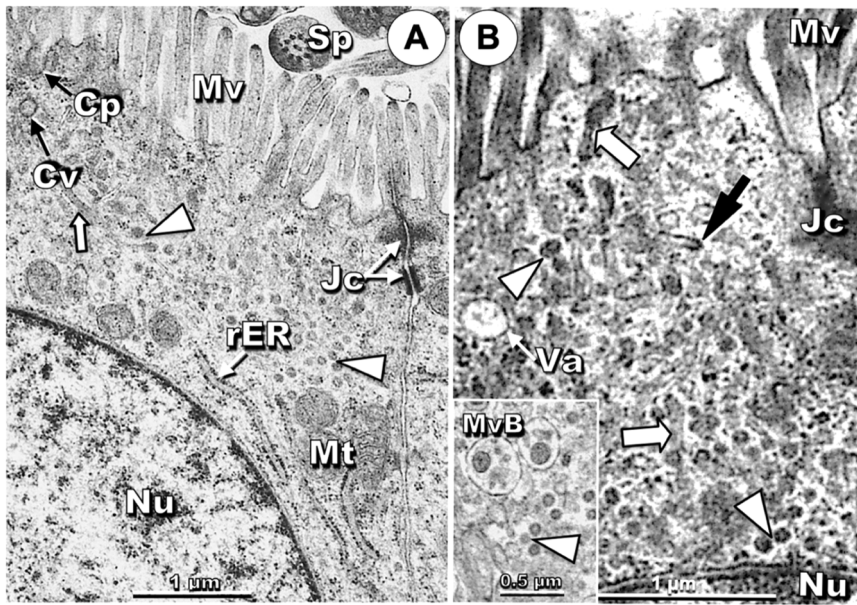


Fig. 19. Sub-Fig. A is a panoramic view of the supra- and para-nuclear regions of the epithelial (NC type III) cell. Sp = sections of spermatozoa in the duct lumen; Mv = microvilli; Jc = apical junctional complex; Cp = coated pit; Cv = coated vesicle; thin block arrow = long profiles of sparsely granulated or smooth surfaced reticulum, giving rise to small vesicles (white arrowheads); Mt = mitochondria; Nu = nucleus; rER = perinuclear strands of rough endoplasmic reticulum. Sub-Fig. B is a higher magnification of the subapical zone of the cell, showing elongated profiles of smooth-surfaced tubules (white arrows), moderately electron-dense transverse sections (white arrowheads) of sparsely or smooth granulated endoplasmic reticulum; black arrow = bristle-coated vesicle budding off a rough endoplasmic reticulum; Jc = junctional complex; Va = vacuole containing sparse, dense material, Nu = nucleus; Mv = microvilli. The inset shows numerous electron-dense vesicles (white arrowhead) and multi-vesicular bodies containing large, dense, oval content (MvB).

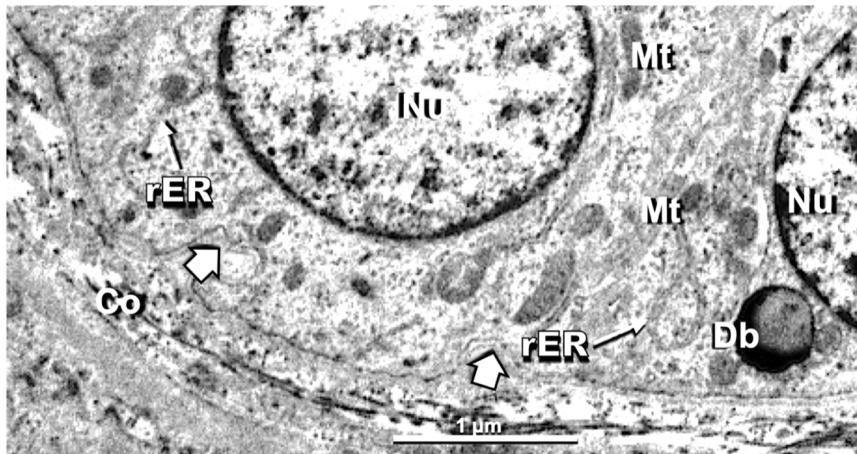


Fig. 20. This is the basal part of the epithelial cells of the connecting and epididymal ducts, showing sparse organelle content. Nu = nucleus; Mt = mitochondria; rER = strands of rough endoplasmic reticulum; Db = lysosome; Block arrow = basal lamina; Co = collagen bundles.

epithelium. In all of the component ducts of the epididymal duct unit of the egret, the baso-lateral desmosomes attaching together adjacent lateral membranes are frequently associated with mitochondria in the so-called 'desmosomal-mitochondrial complexes', which we conveniently shorten to DMC. This phenomenon is discussed further below.

The sub-apical cytoplasm of the principal epithelial cells displayed numerous and more developed and defined elongated, as well as transverse sections of sparsely granulated endoplasmic reticulum than in the earlier duct series. In the egret, the nuclei of the NC III cells in the connecting duct were generally oval or round in shape. This was also the case in the drake and ostrich (Aire, 2002a, 2007), but the nuclei were elongated and in-line aligned with the long axis of the cell in the domestic fowl, turkey and Japanese quail (Tingari, 1972; Hess and Thurston, 1977; and Aire, 2000a). The nuclei of the epithelial cells of the epididymal duct in the egret were relatively large, elongated, and

aligned along the cell axis. The NC III cell is capable of absorbing some luminal content because of the presence of a few coated pits and coated vesicles, and this has been confirmed in the Japanese quail in which the epididymal duct unit reabsorbs a small amount (about 0.8 %) of luminal material from the testis (Clulow and Jones, 1988). The variant of this cell in the ductus deferens, based upon ultrastructural features, is capable of absorbing material from the duct lumen and digesting such material in lysosomes, resulting in the formation of large hetero-lysosomes and telo-lysosomes, that could occupy much of the cytoplasm. This cell, in the ductus deferens, is probably more secretory than absorptive because it has an obvious presence of sparsely granulated or smooth-surfaced endoplasmic reticulum as well as secretory vesicles in the supranuclear region of the cell.

Basal cells have consistently been observed in the epididymal duct unit of birds (Tingari, 1971, 1972; Aire, 1979a; Stefanini et al., 1999;

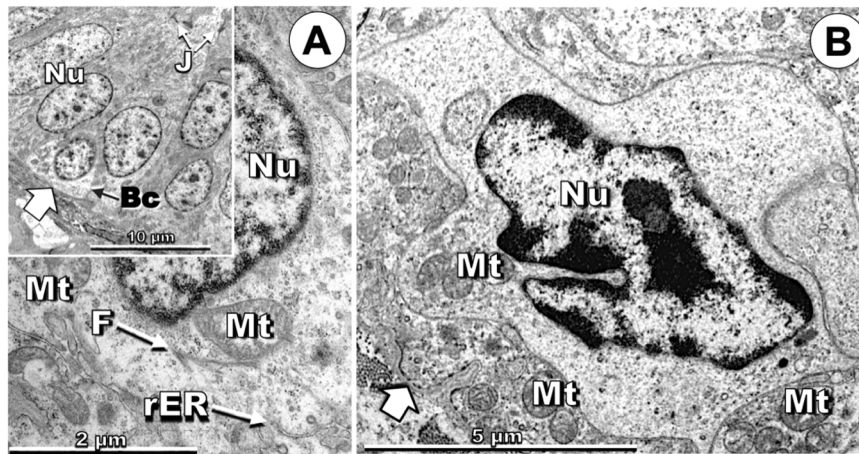


Fig. 21. The inset in Sub-Fig. A shows the epithelium of the ductus deferens displaying a basal cell (Bc) lying on the basal lamina, J = junctional complex, Nu = nucleus, black arrow = basal lamina. The main figure shows the nucleus (Nu) of the basal cell, mitochondria (Mt) and microfilament bundles (F), and a few strands of rough endoplasmic reticulum (rER). Sub-Fig. B shows an electron-lucent cytoplasm of a halo cell, containing very sparse organelle content, a few mitochondria (Mt), and an irregular heterochromatic nucleus (Nu), large block arrow = basal lamina.

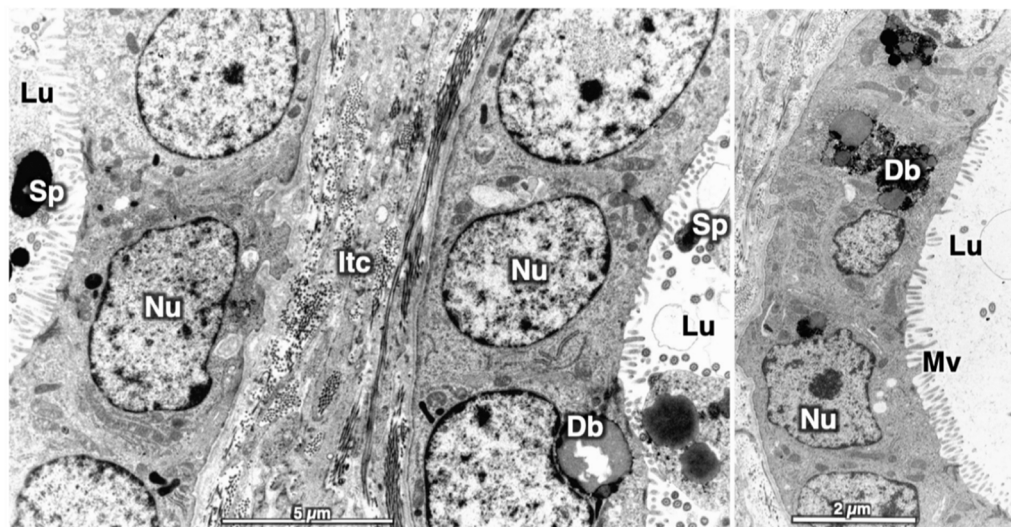


Fig. 22. The figure displays a few profiles of the epithelial lining of the ductus deferens. Lu = lumen; Sp = spermatozoa; Mv = microvilli; Nu = nucleus; Db = dense bodies; Itc = interductal connective tissue.

Aire, 2000a; Aire, 2007) and in all mammals examined (Robaire and Hermo, 1988). There are no reports on the biochemical as well as functional cytology of the basal cells in birds, but it is conjectured that their functions are broadly similar to those in mammals. In mammals, basal cells act as scavengers and luminal sensors in the process of regulating other epithelial cells in the epididymis (Shum et al., 2008; Arrighi, 2014). The nature and functions of the basal cells are still not clearly understood, but they are not progenitors of clear and principal cells during early development in mammals (Clermont and Flannery, 1970; Shum et al., 2008) although they can function as adult stem cells (Croissile et al., 1978; Murashima et al., 2011; Mandon et al., 2015). Further studies on basal cells and immune cells in the excurrent ducts of the testis of birds will undoubtedly contribute to the understanding of their roles in vertebrates, generally.

Halo cells, regarded as intraepithelial cells in reports on birds, have been observed in the excurrent ducts of the testis of several birds (Aire and Malmqvist, 1979b; Aire, 1982a, b; Osman, 1980; Stefanini et al., 1999; Aire, 2000a; Aire and Soley, 2000) and mammals (Dym and Romrell, 1975; Hees et al., 1989). For purposes of comparative biology, the nomenclature of 'halo cell' is adopted in this study. The functions of

halo cells, in various forms, are still not clearly understood, although it is now generally agreed that they have immunological roles in the epididymis. Studies in mammals show that they consist of helper T lymphocytes, cytotoxic T lymphocytes, and monocytes, but not B lymphocytes (Flickinger et al., 1997; Serre and Robaire, 1999), although Wang et al. (2024) consider them to include B lymphocytes. Macrophages, on the other hand, have been seen in the lumen of parts of the excurrent ducts of birds, mainly in the rete testis (Aire and Malmqvist, 1979a; Aire, 1982b; Aire and Josling, 2000), and occasionally have been seen to contain fragments of spermatozoa in normal birds (Aire and Malmqvist, 1979a; Aire and Josling, 2000), unlike in vasectomised birds, in which they appear to be an important route for the elimination, by ingestion, of accumulated and probably damaged spermatozoa (Aire and Heath, 1977; Nakai et al., 1989b; Aire, 2002a).

Rete testis cells in normal species of birds and mammals are occasionally able to remove spermatozoa that are probably damaged or defective (Tingari, 1972; Sinowatz et al., 1979; Holstein, 1978). In this study, the epithelium of the segment of the efferent duct unit, between the proximal and distal ducts showed several fragments of spermatozoa in phagocytic vacuoles, in an apparent attempt to remove damaged or

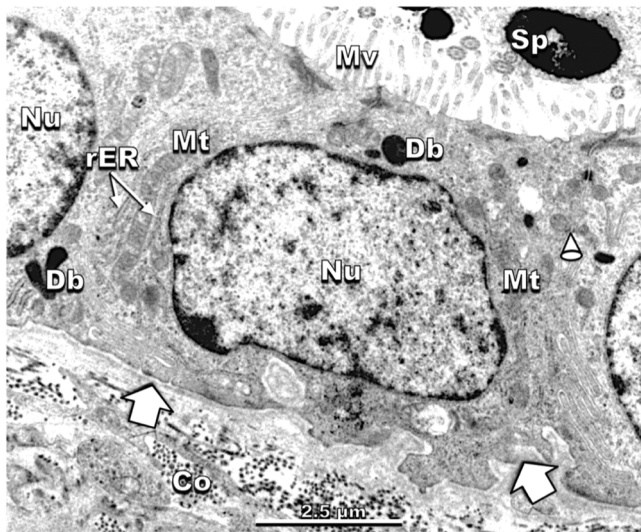


Fig. 23. A low power view of non-ciliated type III cells in a typically cuboidal epithelial lining of the ductus deferens containing large euchromatic nuclei (Nu), a moderate number of mitochondria (Mt), brush borders of microvilli (Mv), which are more sparse than those of the efferent ducts and other segments of the epididymal duct system. Sp = spermatozoa in the duct lumen; Db = dense body; cone 2 = desmosome associated with mitochondria; rER = rough endoplasmic reticulum; block white arrow = basal lamina; Co = collagen bundles.

defective spermatozoa before further progression of the seminal fluid into the more distal segments of the excurrent duct system. Spermiophagy in the initial part of the proximal efferent duct in normal domestic fowl has also been observed (Aire, 2000a).

It is noteworthy that desmosomes between adjacent cells are generally associated with certain mitochondria, usually one only on either side of the desmosome in the egret. The DMCs are present in the efferent ducts where they are few and found between adjacent cells, ciliated or non-ciliated, but they are particularly many and prominent in the NC II and III cells, and much less so in the NC I cells. DMCs are

considered to be a characteristic feature of basic physiological significance of certain epithelia only (Schlotzer-Schrehardt et al., 1990), involving either a single desmosome or a linear array of several desmosomes joined by filamentous bundles associated with one or two mitochondria. Santander et al. (1987) summed up mitochondrial-desmosome complex as “the high-energy, adhering junctional complexes or with mitochondrial coupling, that may be a subtype of the adhering junctions, where the bioenergetics generate electrostatic forces to maintain the intercellular cohesion more effectively and more powerfully, even though this may be of a temporary nature in order to meet, special, functional requirements”. It is also noteworthy that mitochondria within individual cells are morphologically heterogeneous and physically distinct entities (Collins et al., 2002). This indicates that only certain mitochondria in these tissue cells of the egret functioned in this manner, possibly with a view to strengthening the very important blood-epididymis barrier, which, according to Wang et al. (2024), is formed primarily by the baso-lateral and apical membranes of the epididymal principal cells.

Also, certain mitochondria, especially in the basal part of the NC I cells (of the PED) and NC III cells (of the epididymal duct system) of the cattle egret, are closely associated with rER that surround them (Figs. 10 and 22). This association or coupling, that tethers two organelles to each other by means of a protein complex and, thus, creates the physical basis for communication between them, is known as ‘endoplasmic reticulum (ER)–mitochondria encounter structure’, ERMES. Endoplasmic reticulum (ER) and mitochondria are the most important organelles in eukaryotic cells (Zhang et al., 2023). This synergistic link [formed by physical contact points through cholesterol-rich microdomains, called MAMs] enables both organelles to engage in crosstalk with other organelles in their collective or collaborative effort to bring about material transportation, signal transmission, growth, and metabolism (Zhang et al., 2023). The close and special communication between two vital organelles in a cell is necessary for homeostatic control of cellular processes, especially for mitochondria which do not communicate with other organelles through the vesicular trafficking pathway (Xue et al., 2017). The visible expression and relative abundance of the coupling organelles involved in the DMC, as well as those in the ERMES, is noteworthy and appears to be much better developed or more closely examined in the egret than in other avian species studied.

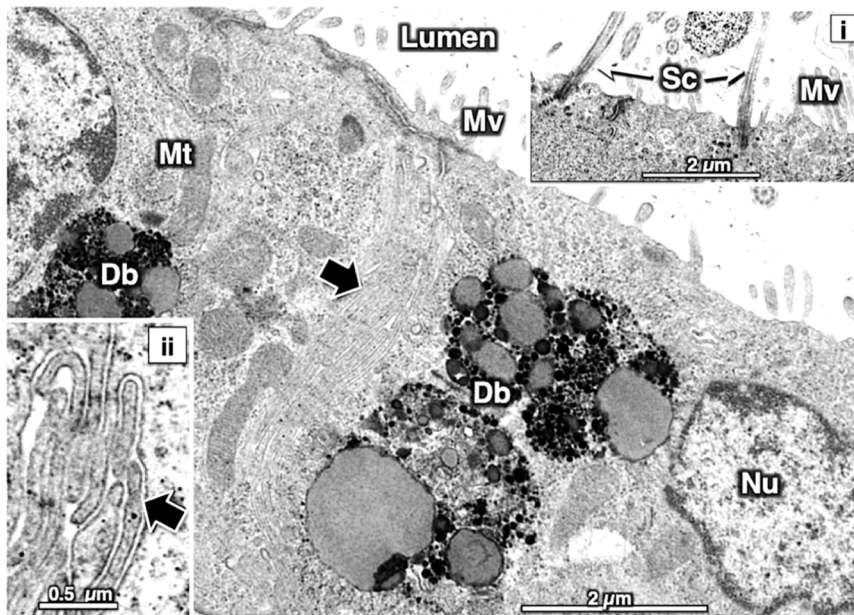


Fig. 24. A low power view of the epithelial lining of the ductus deferens. Inset i: Sc = solitary/primary cilium; Mv = microvilli. Main figure: black broad arrow = complex intertwined intercellular membranes of adjacent cells, enlarged in Inset ii. Mt = mitochondria; Nu = nucleus; Db = dense bodies, specifically, hetero-lysosomes/telolysosomes.

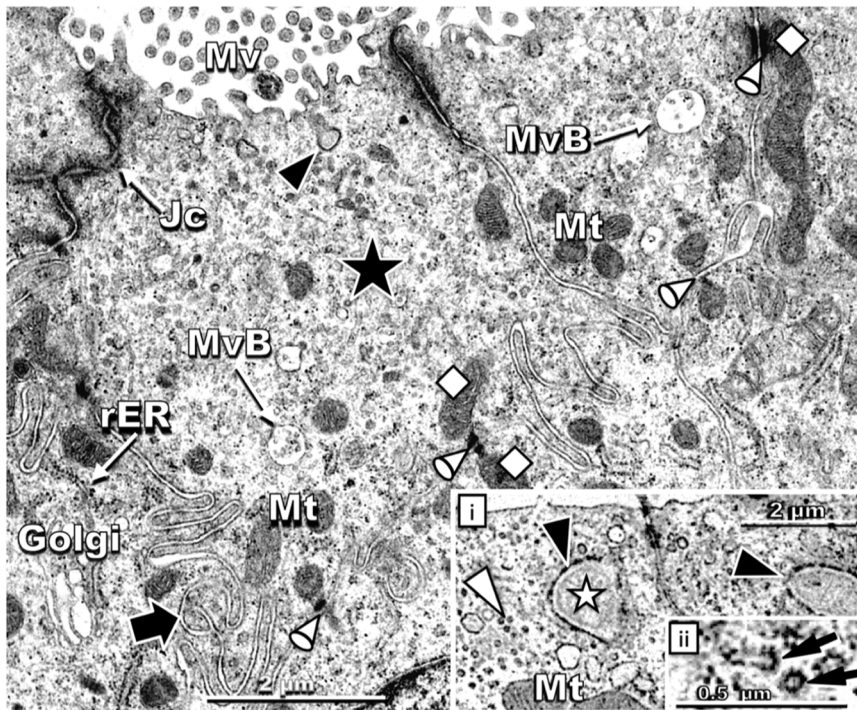


Fig. 25. The main figure shows the supranuclear region of the epithelial lining of the ductus deferens. Mv = microvilli; Jc = junctional complex; cone 2 = desmosome-mitochondrial complex; diamond = mitochondrion involved in the former; black arrowhead = autophagic vacuole; MvB = multivesicular body; Inset i: black arrowhead = autophagosomes containing mitochondria (white star); white arrowheads = electron-dense round vesicles. Inset ii: black arrows = small bristle-coated vesicles.

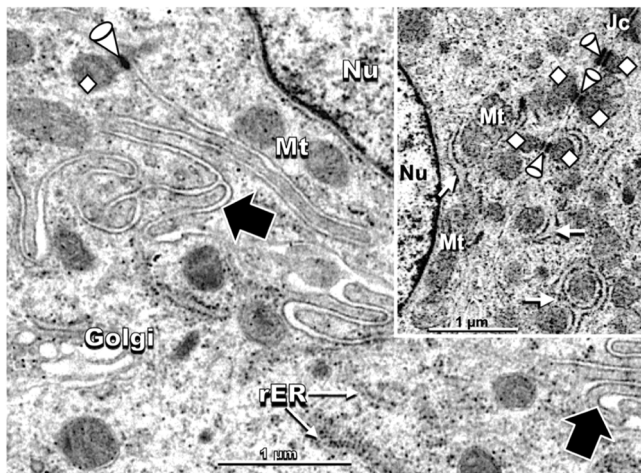


Fig. 26. The basal part of the cell showing complexly intertwined adjacent cell membranes (black block arrows); diamond and cone 2 in a desmosome-mitochondrial complex [DMC]; Mt = free mitochondria; Nu = nucleus. Golgi = Golgi complex; rER = rough endoplasmic reticulum. Inset: white arrows = rER surrounding mitochondria (Mt); cone 2 = desmosomes associated with mitochondria (diamond); Nu = nucleus; Jc = junctional complex.

CRedit authorship contribution statement

Sunil K. Gupta: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Antoinette V. Lensink:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Matthew B.**

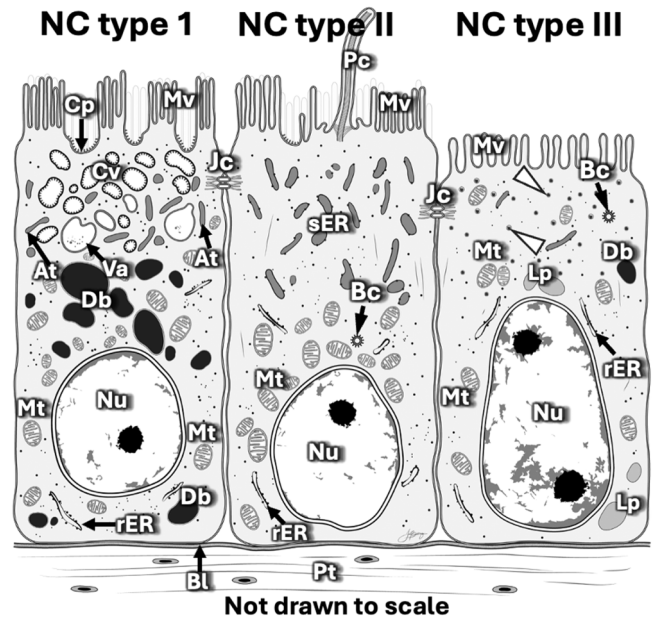


Fig. 27. Schematic representation of the non-ciliated cell types in the excurrent ducts of the testis of the cattle egret. Non-ciliated (NC) type I cell: Mv = microvilli; Cp = coated pits; Cv = coated vesicles; Mt = mitochondria; At = apical tubules; Va = vacuole; Db = dense bodies; rER = rough endoplasmic reticulum; Nu = nucleus. NC type II: smooth and sparsely granulated endoplasmic reticulum in the sub apical zone of the cell; few or no dense bodies/lysosomes; Bc = bristle-coated vesicle; Pc = solitary/primary cilium. NC type III: electron-dense round vesicles (white arrowheads) abound in the sub-apical region of the cell type; Bc = bristle-coated vesicle; Bl = basal lamina; Pt = peritubular tissue.

Charles: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Curtis E. Hopkins:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Roopnarine Narindra:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Tom A. Aire:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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Data availability

Data will be made available on request.

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