Structural and Immunohistochemical Features of the Epididymal Duct Unit of the Ostrich (Struthio camelus)

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With 5 figures and 1 table

ABSTRACT

The epididymal duct unit, comprising the ductus conjugens, ductus epididymidis and ductus deferens, was studied histologically, ultrastructurally and immunohistochemically in five sexually mature and active birds. The main morphological features of the pre-dominant non-ciliated (type III) cell of the epithelial lining of this duct unit include, but are not limited to, a moderately abundant smooth or sparsely granulated endoplasmic reticulum, electron-dense secretory granules and numerous mitochondria in the supranuclear zone of the cytoplasm. A single, large heterogeneous lipid droplet, of unknown function, was characteristically situated immediately proximal to the nucleus. The epithelium is obviously secretory and specifically, of the merocrine, and not apocrine, type of secretion. The epithelium of the epididymal duct unit was only focally and weakly to moderately immunopositive to both actin MF and desmin IF, while the duct unit was immunonegative to cytokeratin and vimentin intermediate filaments. The peritubular muscular layer was moderately to strongly positive to both actin and desmin, and negative to cytokeratins and vimentin.

Introduction

The excurrent ducts of the testis have attracted attention recently because of their role in the maturation, viability and fertility of spermatozoa as they transit through the ducts (Turner, 1991; Hinton and Palladino, 1995; Janssen et al., 1998). Each of the excurrent ducts seems to function in a specific or complementary role, as they, collectively, create a number of microenvironments through which the spermatozoa pass (Turner, 1991). For example, the reabsorption of most of the testicular fluid that enters the duct system occurs in the efferent ducts in mammals and birds (Crabo, 1965; Clulow and Jones, 1988). It is now established that this function of the efferent ducts is indispensable for further maturation and viability of spermatozoa in the mouse (Hess et al., 1997). Although not much is known about the specific roles of the epididymal duct and ductus deferens in birds, about four Wolffian duct proteins bind to spermatozoa as they pass through the epididymis and ductus deferens of the rooster (Esponda and Bedford, 1985; Morris et al., 1987). It is noteworthy that epididymal proteins are order-specific in birds (Esponda and Bedford, 1985; Morris et al., 1987).

The epididymis of birds comprises a maze of ducts, including the rete testis, efferent duct or ductuli efferentes, connecting duct or ductus conjugens and epididymal duct or ductus epididymidis. 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, 1979) 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 ducts be collectively known as the epididymal duct unit, in birds. This duct unit constitutes about 74% of the entire excurrent duct volume in the Japanese quail (Clulow and Jones, 1988). In a preliminary study, on the ostrich epididymis, both the connecting and epididymal ducts

account for about 26% of the epididymal volume, as against about 10% for the domestic fowl, 4% for the Japanese quail and 9% for the turkey (Aire, 2007). The significance of this great disparity in duct volumetric proportion in favour of the ostrich is not understood. The rete testis, efferent ducts and epididymal duct unit of mammals demonstrate actin microfilaments, as well as cytokeratin, desmin and vimentin intermediate filaments, both in the ducts and in the periductal smooth muscle cell layer. The immunohistochemistry of microfilaments and intermediate filaments in the reproductive organs and tracts of mammals have received recent attention, but their roles are still speculative (Kasper and Stosiek, 1989; Dinges et al., 1991 and Wakui et al., 1994). However, the normal structural and immunohistochemical features, other than the structural features of the rete testis (Aire and Soley, 2003) and the efferent ducts (Ozegbe et al., 2006), of the epididymis of the ostrich have not been determined. This paper sought to contribute to filling this gap in our knowledge, especially in a primitive and highly economic bird, such as the ostrich whose low fertility is a concern to ostrich farmers (Deeming and Ar, 1999).

Materials and Methods

Tissues of the epididymis as well as ductus deferens from five sexually mature and active ostriches slaughtered at local abattoirs were fixed by immersion in either Bouin's fluid or 10% buffered formalin or 3% glutaraldehyde buffered in either Millonig's buffer or sodium cacodylate buffer. The Bouin's fluid and formalin-fixed tissues were processed for light microscopy, using standard, conventional methods. Appropriate glutaraldehyde-fixed tissue blocks were subsequently processed by standard methods, through secondary fixation in osmium tetroxide, dehydration through a graded series of ethanol, and subsequent embedment in resin. Sections of 1 µm thick were cut and stained with toluidine blue for light microscopy. Ultra-thin sections were cut, stained conventionally with uranyl acetate and lead citrate and photographed in a Philips electron microscope. The Bouin's fluid-fixed as well as the formalin-fixed tissues were processed routinely and embedded in paraffin wax, for histology and immunohistochemistry, respectively.

For immunohistochemistry, sections of 5 µm thick of formalin-fixed, and paraffin-embedded tissues were cut and mounted on slides pre-coated with polylysine, deparaffinized, rehydrated and then heat-treated for antigen retrieval. The sections were incubated for 5 min in hydrogen peroxide (3% in distilled water) to reduce endogenous peroxidase activity. The slides were then rinsed in a 0.01 m phosphate buffer saline solution (PBS, pH 7.4), containing bovine serum albumen, for 5 min to block non-specific binding sites. Immunostaining, for 30 min at room temperature, using the LSAB-plus kit (Dakocytomation, Glostrup, Denmark) monoclonal antibodies against smooth muscle actin, cytokeratin, desmin and vimentin (at dilutions of 1:50, 1:100, 1:300 and 1:100, respectively) was performed on the slides. Thereafter, the slides were rinsed in PBS. Biotinylated secondary antibody (LSAB-plus kit; Dakocytomation) was applied to the sections for 15 min in a humidified chamber at room temperature, and followed by streptavidin component of the LSAB-plus staining kit for another 15 min. Immunoreactivity was visualized after the addition of a substrate solution, NovaRED™ (Substrate kit SK-4800; Vector® Laboratories Inc., Burlingame, CA, USA) and sections were counter-stained with haematoxylin. Negative controls involved the primary antibody replaced by bovine serum albumen. Smooth muscle was used as a positive control for both desmin and smooth muscle actin, whilst tonsillar tissue and skin were used as positive controls for vimentin and cytokeratin, respectively.

Results and Observations

Histology and ultrastructure

The connecting ducts are found in scattered foci throughout transverse sections of the epididymis, and their epithelial height varies considerably. The epididymal duct occupies the medial border of the epididymis, and is wavy in outline. The ductus deferens extends this unit

distally, as it courses caudally, in progressive waviness and increasing duct diameter. The columnar epithelium of all three segments of this duct unit comprises non-ciliated cells (non-ciliated type III cells of Aire, 1979) and basal cells (Fig. 1). The euchromatic nuclei of the columnar cells are generally oval to elongate in shape and contain one or two free nucleoli.

Ultrastructurally, the non-ciliated (NC type III) cell (Aire, 1979) is very long and narrow, with the width of both the nucleus and cell being very close (Fig. 1). Most of the cytoplasm of the cell is supranuclear (Figs 1 and 2). The relatively euchromatic nucleus is elongated along the long axis of the cell, is situated towards the basal part of the cell. A single relatively large, heterogeneous lipid droplet regularly occurs, just proximal to the nucleus (Figs 1 and 2).

There is a moderate abundance of profiles of smooth endoplasmic reticulum (SER) and sparsely granulated endoplasmic reticulum (SGER) throughout the cytoplasm in the supranuclear region of the cell (Fig. 2). Occasionally, a NC cell displays more profiles of dilated SER and SGER containing a flocculent material, than are to be found in most of the other cells (Fig. 2). Profiles of SER and SGER are few in the infranuclear parts of the NC cells (Fig. 4). Profiles of rough endoplasmic reticulum (RER), in their characteristic features are few and inconspicuous. The Golgi complex, in the supranuclear region, is very well developed and comprises four saccules (Fig. 2) which, on the trans-Golgi zone bud off several vesicles containing electron-dense material (Figs 2 and 3). Several dense, round/oval, membrane-bound bodies, akin to secretory vesicles, are scattered throughout the supra-Golgi region of the cytoplasm. A number of smooth-walled vesicles containing inspissated electron-dense content may also be seen in the subapical zone of the cell (Fig. 3). Several of these two types of vesicles seem to approach the apical plasmalemma. Mitochondria of varying profiles are moderately numerous in the supranuclear and infranuclear regions, and their cristae are embedded in a dense matrix (Figs 2–4). The apical cell surface bears numerous, regular, short microvilli, and a few apical pits may be seen (Figs 2 and 3). The lateral plasmalemma is usually regular, and only slightly folded basally (Fig. 4).

Basal cells, usually rest on the basal lamina, and are surrounded by one or more NC cells. They increase in number proximo-distally, being, therefore, most numerous in the ductus deferens. The nuclei of these cells are longitudinally elongated and irregular in shape. The organelle content of the cell is sparse and consists, mainly, of small mitochondria, a small Golgi complex, a few, thin strands of RER and bundles of microfilaments/fibrillar material (Fig. 4). A number of intraepithelial lymphocytes were also observed in the epithelium (Fig. 1).

Immunohistochemistry

Generally, vimentin and cytokeratin intermediate filaments were immunonegative in the various segments of the epididymal duct unit as shown in Table 1. The epithelium, as well as the inner layer of the periductal tissue and tunica intima, was also immunonegative to actin and desmin (some areas in the epithelium were, at best, very weakly positive for desmin) intermediate filaments. The outer, but not the one or two innermost cell, layers of the periductal tissue showed slight to moderate immunopositivity to actin intermediate filament (Fig. 5a and Table 1).

The spermatozoa, in all the ducts, including the epididymal unit ducts, were moderately to strongly immunostained to actin. The capsular tissue of the epididymal duct unit showed two degrees of actin immunopositivity: sparsely immunostained bundles and strongly immunostained strands of tissue. The tunica adventitia of the blood vessels was also strongly immunopositive to actin as shown in Fig. 5a and Table 1.

There was a weak to moderate positive immunoreaction to desmin in the outer layer of the periductal tissue (Fig. 5b and Table 1). The capsular tissue of the epididymal duct unit showed scattered bundles of moderately to strongly immunopositive reaction for desmin while those of the tunica adventitia of the various blood vessels (Fig. 5c) were uniform and strong.

Discussion

The projection of regular, moderately long microvilli and a solitary cilium from the surface of the NC type III cell of the ostrich (Aire and Soley, 2000) is similar to that described for some other birds (Aire, 1982; Aire and Josling, 2000), but adjacent membranes are relatively simple and are not as intricately and complexly folded as in domestic anseriform and galliform birds (Aire, 2000).

The cytoplasmic organelle content suggests a secretory role for the NC type III cell of the ostrich because of the presence of moderately abundant SER and SGER, a moderately developed but extensive Golgi complex, and secretory vesicles in the sub-apical zone of the cell. As in other birds (Aire, 2000), the Golgi complex of the ostrich displays only four saccules, unlike in rats which generally have about eight saccules (Hermo et al., 1991). The significance of this differential in number of saccules between mammals and birds, with regard to function of this organelle, is not clearly understood. The endoplasmic reticulum of the cell in the ostrich is similar to that seen in the drake, and which exhibits a predominance of SGER. Dense granules, probably secretory granules in the cell of the ostrich are, however, similar to those described for the domestic fowl by Tingari (1972), in having a homogeneously dense content, but dissimilar to those of the drake that contain centrally located, apparently inspissated, secretory material (Aire, 2000). The presence of usually few subapical vesicles containing centrally-located inspissated material in the ostrich suggests that this cell may produce, at least, two types of secretions of varying composition and density. They could, however, be absorptive vesicles. In the Japanese quail and turkey, secretory vesicles are not a prominent feature in this cell. Instead, microtubules are well developed and conspicuous in the area proximal to the Golgi complex. Microtubules are now known to play an active role in the transport of cellular secretory products (Darnell et al., 1990), and could be particularly important in cells that omit the concentration of secretory material, and, thus, lack secretion granules (Palade, 1975). The epididymal duct unit of the ostrich, as in other birds studied (Tingari, 1972; Hess and Thurston, 1977; Aire, 2000), may have a minimal role in fluid absorption as has been determined in the Japanese quail (Clulow and Jones, 1988).

The presence of a solitary, heterogeneous lipid droplet in the immediate supranuclear region of the cell has also been reported in the rete testis, as well as the efferent ducts, of the ostrich (Aire and Soley, 2003; Ozegbe et al., 2006). The significance of this droplet is not known, but it may be regarded as a species-characteristic feature of these non-ciliated cell types in the ostrich. Basal cells are present only in the epididymal duct unit of the excurrent ducts of birds, and they increase in number distally. Their functions in the epididymal duct of animals are not clearly understood, but recent studies have shown that they may be involved in a number of processes in the epithelium, including but not limited to, immunological functions, being tissue-fixed macrophages in the epithelium (Seiler et al., 1998, 2000; Holschbach and Cooper, 2002) and the ability to grow around and, thus, sequester spermatozoa from a damaged epithelium (Aruldhas et al., 2004).

Smooth muscle actin and desmin intermediate filaments are found in contractile cells (Van Nassauw et al., 1993). Both actin and desmin intermediate filaments have only recently been

described in the excurrent ducts of the testis of the domestic fowl (Maretta and Marettová, 2004). The immunohistochemical results indicate that actin MF and desmin IF are focally and only moderately immunoexpressed in the epithelium of both the connecting and epididymal ducts. The absence of actin activity in the epithelium of the excurrent ducts of the testis was reported by Maretta and Marettová (2004) in the domestic fowl. However, in mammals, Achtstätter et al. (1985), Kasper and Stosiek (1989), Dinges et al. (1991) and Palacios et al. (1993) in humans, and Wakui et al. (1994) in the dog, have demonstrated cytokeratins and vimentin in the epididymal epithelium. It is not known, if these differences in the presence of IFs in epithelial cells of the epididymal duct between mammals and the ostrich are related to differences in cellular function.

A moderate to strong presence of actin and desmin, but not cytokeratin and vimentin, in the interductal tissue in the ostrich, is, also, in consonance with observations made in the domestic fowl by Maretta and Marettová (2004). The contractile cells in this tissue appear to be necessary for onward propulsion of spermatozoa from both the epididymal duct unit and the epididymis, as a whole.

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References

- Achtstätter, T., R. Moll, B. Moore, and W. W. Franke, 1985: Cytokeratin polypeptide patterns of different epithelia of the human male urogenital tract. J. Histochem. Cytochem. 33, 415–426. Links
- Aire, T. A., 1979: The epididymal region of the Japanese quail (Coturnix coturnix japonica). Acta Anat. 103, 305–312. Links
- Aire, T. A., 1982: Surface morphology of the ducts of the epididymal region of the drake (Anas platyrhynchos) as revealed by scanning and transmission electron microscopy. J. Anat. 135, 513–520. Links
- Aire, T. A., 2000: Aspects of the functional morphology of the ductus epididymidis in domestic anseriform and galliform birds. Anat. Histol. Embryol. 29, 171–191. Links
- Aire, T. A., 2007: Anatomy of the testis and male reproductive tract. In: Reproductive Biology and Phylogeny of Birds. Vol. 6A (B. G. M. Jamieson, ed.). New Hampshire, USA, Plymouth, UK: Science Publishers Inc., pp. 37–113.
- Aire, T. A., and D. Josling, 2000: Ultrastructural study of the luminal surface of the ducts of the epididymis of gallinaceous birds. Onderstepoort J. Vet. Res. 67, 191–199. Links
- Aire, T. A., and J. T. Soley, 2000: The surface features of the epithelial lining of the epididymis of the ostrich. Anat. Histol. Embryol. 29, 119–126.

Links
- Aire, T. A., and J. T. Soley, 2003: The morphological features of the rete testis of the ostrich (Struthio camelus). Anat. Embryol. 207, 355–361. Links
- Aruldhas, M. M., S. Subramanian, P. Sekhar, G. C. Hasan, P. Govindarajulu, and M. A. Akbarsha, 2004: Microrecanalization in the epididymis to overcome ductal obstruction caused by chronic exposure to chromium – a study in the mature bonnet monkey (Macaca radiata Geoffroy). Reproduction 128, 127–137. Links
- Clulow, J., and R. C. Jones, 1988: Studies of fluid and spermatozoal transport in the extratesticular ducts of the Japanese quail. J. Anat. 157, 1–11. Links
- Crabo, B., 1965: Studies on the composition of epididymal content in bulls and boars. Acta Vet. Scand. 6(Suppl. 5), 8–94. Links
- Darnell, J., H. Lodish, and D. Baltimore, 1990: Microtubules and cellular movements. In: Molecular Biology (J. Darnell, H. Lodish and D. Baltimore, eds). New York: Scientific American Books, pp. 815–858.
- Deeming, D. C., and A. Ar, 1999: Factors affecting the success of commercial incubation. In: The Ostrich: Biology, Production and Health (D. C. Deeming, ed). Wallingford: CABI Publishing, pp. 159–190.
- Dinges, H. P., K. Zatloukal, C. Schmid, S. Mair, and G. Wirnsberger, 1991: Co-expression of cytokeratin and vimentin filaments in rete testis and epididymis. Virchows Arch. A Pathol. Anat. 418, 119–127. Links
- Esponda, P., and J. M. Bedford, 1985: Surface of the rooster spermatozoon changes in passing through the Wolffian duct. J. Exptal. Zool. 234, 441–449. Links
- Hermo, L., H. Green, and Y. Clermont, 1991: Golgi apparatus of epithelial principal cells of the epididymal initial segment of the rat: structure, relationship with endoplasmic reticulum, and role in the formation of secretory vesicles. Anat. Rec. 229, 159–176. Links
- Hess, R. A., and R. J. Thurston, 1977: Ultrastructure of the epithelial cells in the epididymal region of the turkey (Meleagris gallopavo). J. Anat. 124, 765–778. Links
- Hess, R. A., D. Bunick, K. H. Lee, J. Bahr, J. A. Taylor, K. S. Korach, and D. B. Lubahn, 1997: A role for estrogens in the male reproductive system. Nature 390, 509–512. Links
- Hinton, B. T., and M. A. Palladino, 1995: Epididymal epithelium: its contribution to the formation of a luminal fluid microenvironment. Microsc. Res. Tech. 30, 67–81. Links
- Holschbach, C., and T. G. Cooper, 2002: A possible extratubular origin of epididymal basal cells in the mice. Reproduction 123, 517–525. Links
- Janssen, S., D. Bunick, C. Finnigan-Bunick, Y. C. Chen, R. A. Hess, and J. Bahr, 1998: Morphology and function of rooster efferent ductule epithelial cells in culture. Tissue Cell 30, 554–561. Links
- Kasper, M., and P. Stosiek, 1989: Immunohistochemical investigation of different cytokeratins and vimentin in the human epididymis from the fetal period up to adulthood. Cell Tissue Res. 257, 661–664. Links
- Maretta, M., and E. Marettová, 2004: Immunohistochemical demonstration of myoid cells in the testis and its excurrent ducts in the domestic fowl. Br. Poult. Sci. 45, 585–589. Links
- Morris, S. A., B. Howarth Jr, J. W. Crim, S. Rodriguez de Cordoba, P.

Esponda, and J. M. Bedford, 1987: Specificity of sperm-binding Wolffian duct proteins in the rooster and their persistence on spermatozoa in the female host glands. J. Exptal. Zool. 242, 189–198. Links

- Ozegbe, P. C., T. A. Aire, and J. T. Soley, 2006: The morphology of the efferent ducts of the testis of the ostrich, a primitive bird. Anat. Embryol. 211, 559–565. Links
- Palacios, J., J. Regadera, R. Paniagua, C. Gamallo, and M. Nistal, 1993: Immunohistochemistry of the human ductus epididymis. Anat. Rec. 235, 560–566. Links
- Palade, G., 1975: Intracellular aspects of the process of protein synthesis. Science 189, 347–358. Links
- Seiler, P., I. Wenzel, A. Wagenfeld, C.-H. Yeung, E. Nieschlag, and T. G. Cooper, 1998: The appearance of basal cells in the developing murine epididymis and their temporal expression of macrophage antigens. Int. J. Androl. 20, 217–226. Links
- Seiler, P., T. G. Cooper, and E. Nieschlag, 2000: Sperm number and condition affect the number of basal cells and their expression of macrophage antigen in the murine epididymis. Int. J. Androl. 23, 65–76. Links
- Tingari, M. D., 1971: On the structure of the epididymal region and ductus deferens of the domestic fowl (Gallus domesticus). J. Anat. 109, 423–435. Links
- Tingari, M. D., 1972: The fine structure of the epithelial lining of the excurrent duct system of the testis of the domestic fowl (Gallus domesticus). QJ. Exp. Physiol. 57, 271–295. Links
- Turner, T. T., 1991: Spermatozoa are exposed to a complex microenvironment as they traverse the epididymis. In: The Male Germ Cell, Spermatogonium to Fertilization, Vol. 637, (B. Robaire, ed.). New York: Acad. Sci., pp. 364–384.
- Van Nassauw, L., F. Harrison, and M. Callebaut, 1993: Smooth muscle cells in the peritubular tissue of the quail testis. Eur. J. Morphol. 31, 60–64. Links
- Wakui, S., M. Furusato, S. Ushigome, and Y. Kano, 1994: Coexpression of different cytokeratins, vimentin and desmin in the rete testis and epididymis of the dog. J. Anat. 184, 147–151. Links

Table 1. Presence and intensity of immunolabeling of intermediate filaments in the epididymal duct of the ostrich

	Actin	Cytokeratin	Desmin	Vimentin
Epithelium periductal tissue	–ve to + (foci)	–ve	+/$_2$ to –ve	–ve
Inner cell layers	–ve	–ve	–ve	–ve
Outer cell layers	+ + +	–ve	+ + to + + +	–ve
Blood vessels				
Tunica adventitia	+ + +	–ve	+ + +	–ve
Tunica intima	–ve	–ve	–ve	–ve
Spermatozoa	+ + to + + +	–ve	+/$_2$ to +	–ve

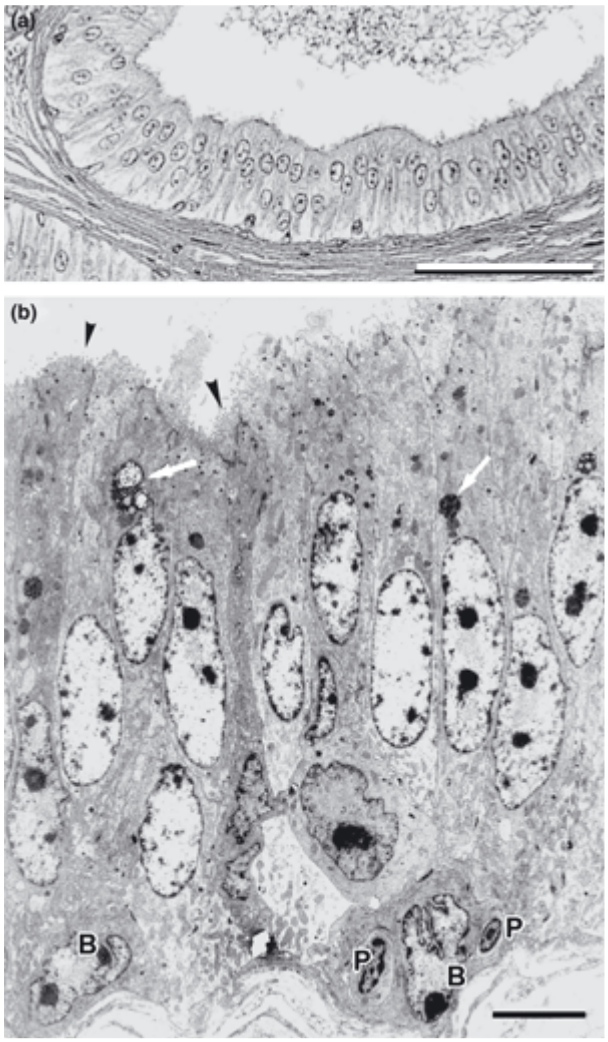

Fig. 1. (a) The epithelium of the connecting duct is simple columnar and may be pseudostratified. The epithelial nuclei generally exhibit two nucleoli, and the duct is supported by a thick peritubular connective tissue. H & E stain. (b) A survey electron micrograph of the epididymal duct shows tall, columnar, principal, non-ciliated (type III) cells displaying short apical microvilli (arrowheads), basal cells (B) and intraepithelial lymphocytes (P). Dense, heterogeneous lipoid droplets (arrows) may be seen in the immediate supranuclear region of the principal cell whose nucleus is nearly as wide as the cell. Bars: a) 50 µm, b) 5 µm.

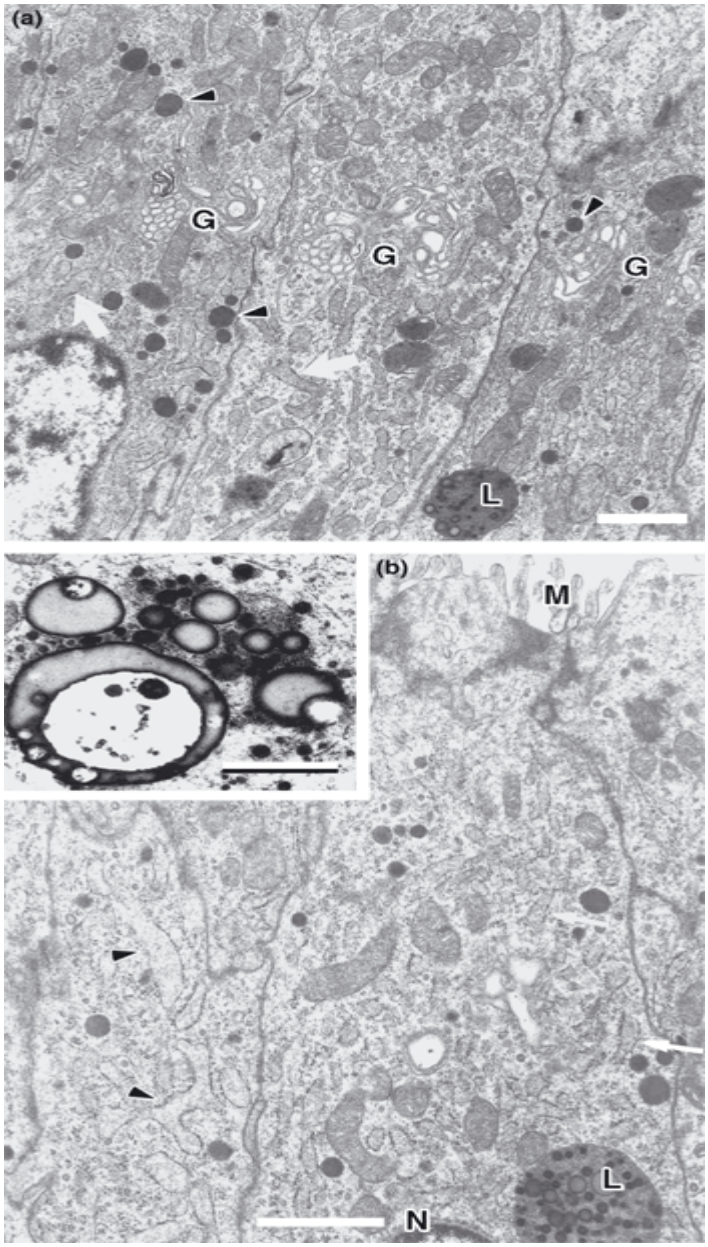

Fig. 2. The supranuclear region of the principal cell cytoplasm is extensive, and contains important cell organelles. (a) The Golgi complex (G) is well developed and occupies the supranuclear part of the principal cell. Numerous, uniformly dense, small, membrane-bound granules (arrowheads) are found in the supranuclear zone of the cell, up to the apical cell surface. A moderate abundance of smooth endoplasmic reticulum (SER)/sparsely granulated endoplasmic reticulum (SGER) (arrowheads) is evident in the cell. The lateral cell membrane is wavy or only moderately folded at this level. L, lipid droplet. (b) Profiles of SER/SGER (arrowheads) are more dilated and branched in the cell on the left than those in the right cell in which the organelle is of smaller, non-branched shapes (arrows). L, a heterogeneous lipid droplet lying close to the nucleus (N); M, short, regular microvilli. Inset: a high power view of a heterogeneous lipid droplet displaying varying configurations in the droplet. Bars: a) and b) 1 µm; Inset on b) 0.5 µm.

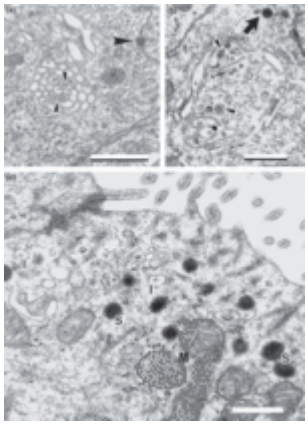

Fig. 3. (a) and (b) show higher power view of parts of the Golgi complex in the principal cells of the epididymal duct. (a) Golgi saccules arranged in a whorl-like manner exhibit concentrated secretory material (small arrowheads) in the central portion of the whorl. The large arrowhead = desmosome junctional complex of adjacent cell membranes. (b) Numerous secretory vesicles containing dense material (arrowheads) are budded off the trans side of the Golgi apparatus. Arrow = a secretory granule and an adjacent, smaller clathrin-coated vesicle. (c) A higher power view of the subapical zone of a principal cell. Uniformly dense secretory granules (S) and other vesicles, probably secretory vesicles also, containing inspissated material (I) are found at this level in the cell. Mitochondria (M) are numerous and contain a dense matrix. Bars: a) 1 μm, b) 0.5 μm, c) 5 μm.

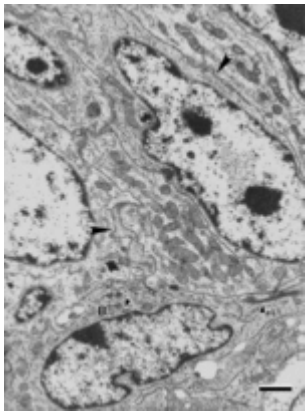

Fig. 4. The basal part of the epithelium of the epididymal duct unit. Mitochondria are mostly vertically oriented and numerous in the infranuclear part of the principal cells. Sections of smooth endoplasmic reticulum/sparsely granulated endoplasmic reticulum are fewer in number and smaller in size than in the supranuclear area. Dense secretory granules are also absent in this part of the cell. A basal cell (B) displays an irregular, horizontally-oriented nucleus. The organelle content is sparse, but fibrillar bundles (small arrowheads) are a characteristic feature of this cell. Adjacent cell membranes (large arrowheads) may be slightly folded only. Bar, 1 μm.

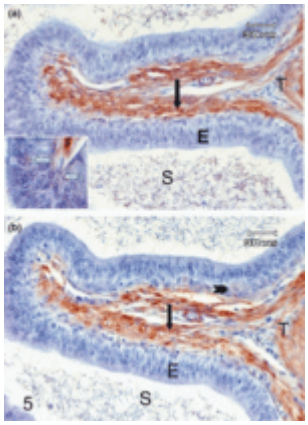

Fig. 5. (a) The connecting and epididymal duct epithelium (E) is generally immunonegative to actin, but a few a foci (inset) are immunopositive, at higher magnification. The periductal smooth muscle layer (long arrows) and the tunica adventitia (T) are strongly and moderately positive for actin and desmin, respectively. Spermatozoa (S) in the ductal lumen are moderately to strongly immunopositive to actin. (b) The connecting duct of the ostrich shows moderately to strongly desmin immunoreactivity in the periductal muscular layer (long arrow), but the E which is generally immunonegative, displays a few foci (probably macrophages or intraepithelial lymphocytes), of slight desmin immunoreactivity (thick arrowhead). The periductal connective tissue (T) is moderately positive to desmin. S in the ductal lumen is weakly immunopositive to desmin.