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HISTOLOGY, FINE STRUCTURE AND CARBOHYDRATE HISTOCHEMISTRY OF THE EPITHELIUM OF THE ACCESSORY MALE GENITAL GLANDS OF THE EGYPTIAN BALADI GOAT

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SUMMARY

The histology and carbohydrate content of the epithelium of accessory male genital glands of Baladi goats in Egypt have been studied by light and electron microscopy. The histochemical methods include periodic acid-Schiff and alcian blue stain (pH 2.5 and 1.0). The results revealed that the prostatic secretory end-pieces are lined with cuboidal cells and their cytoplasm contain fine secretory granules that have an affinity for PAS and alcian blue. In some regions, the secretory cells are columnar with PAS-positive nonalcianophilic cytoplasm. The results indicated that the prostate glands are involved in the production of acid mucosubstances besides little amounts of neutral glycoproteins. Similar patterns were demonstrated in the bulbo-urethral glands and seminal vesicles except that there were relatively fewer or no neutral carbohydrates in the cytoplasm of the bulbo-urethral and vesicular epithelial cells. The acidic carbohydrates consist of at least two types: AB (pH 1.0)-reactive sulfated and AB (pH 2.5)-reactive nonsulfated. Unlike the other accessory genital glands, the secretory tubules of the ampullary glands contained a variable number of PAS-positive secretory granules indicating its neutral glycoprotein nature. A considerable number of spermatozoa was observed within the lumina of some secretory tubules of the ampullary glands. A finding might indicate that the epithelial cells of the ampullary glands are involved in development and maturation of the epididymal spermatozoa. The histochemical and fine structure of the goat accessory male genital glands indicate that their cells could be regarded as glycoprotein secreting cells and their secretory products are crucial for the normal function of sperms.

Key Words: Light microscopy; electron microscopy, histochemistry; prostate gland; seminal vesicles; bulbo-urethral glands; ampullary glands; goat

INTRODUCTION

Goats in Egypt are promising sources for meat and milk and are expected to provide significant quantities of animal proteins in the coming decades. Efficient reproduction in buck is to a large extent dependent on effective functions of genital glands. The accessory genital glands of the buck include the prostate, bulbo-urethral, and ampullary glands as well as seminal vesicles. The epithelial lining of these glands is the source of many biologically active substances that provide the spermatozoa with the proper milieu in which they can survive and attain a powerful fertilizing capability (Chan and Wong, 1998; El-Alfy et al., 2000 and Arenas et al., 2001).

Based on their secretory activities, the epithelial cells of accessory male genital glands were found to be of three types: (a) mucus-producing cells, (b) protein-secreting cells, and (c) seromucoid producing cells. All cells were found to contain variable amounts of neutral and acid carbohydrate complexes (Tsukise and Yamada, 1984).

Studies on the accessory male genital glands of different animals have concentrated on the structural changes in response to seasonal variation e.g., mice (Kruczek and Gruca 1990 and Demas and Nelson, 1998); Reeve's muntjac (Chapman and Harris, 1991); rodents (Gernigon et al., 1994); rat's (Gottreich et al., 1996); rams (Pinckard et al., 2000); calves (Aravindakshan et al., 2000) and camels (Al-Qarawi et al., 2000).

The authors could not come across detailed description of the histology and carbohydrate histochemistry in the epithelium of accessory male genital glands of Baladi goats in Egypt. Hence, the present study was therefore designed to fulfill this gap.

MATERIALS AND METHODS

The materials used in the present study were obtained from 30 sexually mature apparently healthy Baladi bucks. The animals ranged between 1-2.5 years. After slaughtering, the different accessory genital glands were processed for routine histological examination. The tissues were fixed in 10 % buffered neutral formalin and Bouin's solution, dehydrated and embedded in paraffin. Sections of 5-6 µm thick were prepared and stained with Harris Hematoxylin and Eosin for general studies; Crossman's trichrome and Van-Giesonís stains as differential stains; Periodic acid Schieff (PAS) technique for demonstration of neutral mucopolysaccharides; combination of PAS and AB (pH 2.5) for localization of mucosubstances: alcian blue (pH 1 and 2.5) for differentiation of sulfated and nonsulfated acid mucopolysaccharides, alcian blue-Van-Gieson's combination as well as Best's

carmine for the detection of glycogen. The histological and histochemical staining methods were conducted as outlined by Carson (1990).

For electron microscopy, selected pieces from the different glands were fixed for 12 hours in 2.5% glutaraldehyde in cacodylate buffer adjusted to pH 7.4. After rinsing with the buffer, the tissues were postfixed in 1% buffered osmium tetroxide for 2.5 h at 4 °C. The specimens were then quickly rinsed in the buffer, dehydrated in up graded series of ethanol, and were embedded in poly Bed resin Carson, (1990) . Semithin sections were cut and stained with 1% toluidine blue. Thin sections were cut and mounted on copper grids and double stained with uranyl acetate and lead citrate and examined with JEOL 100c transmission EM at 80 kv.

RESULTS

Prostate glands

The prostatic secretory end-pieces were tubulo-alveolar with wide lumina (Plate 1, a & b). The secretory cells were cuboidal to columnar in shape and their cytoplasm was acidophilic (Plate 1, b) and stainable with PAS (Plate 1, c) and alcian blue (Plate 1, d). The comparatively small spherical or oval nuclei were located onto the basal lamina (Plate 2, a). In some regions, the secretory cells were columnar with PAS-positive non-alcianophilic cytoplasm and their oval nuclei were located basely (Plate 2, b). Intralobular ducts lined by simple cuboidal, PAS-negative and nonalcianophilic cells were observed (Plate 2, c). The ducts merged with each other to form a larger interlobular duct lined with columnar (Plate 2d) nonalcianophilic PAS-negative cells. The main excretory duct was lined with cuboidal or stratified cuboidal PAS-negative nonalcianophilic epithelium. It opens into the prostatic urethra.

In electron micrographs, the columnar secretory cells contained oval to elongated, indented, basal and euchromatic nuclei (Fig. 1) and the cell's cytoplasm showed well-developed elements of rER, Golgi apparatus and lysosome-like dense bodies (Fig. 2).

Bulbo-urethral glands

The bulbo-urethral glands of the Baladi goats were composed of branched tubular or tubulo-alveolar end-pieces which were organized into lobules. The interlobular septa were collagenous and contained smooth muscle fibers (Plate 3, a, b). The secretory end-pieces were large with wide lumina (Plate 3, b). The secretory cells were cuboidal to pyramidal and the cytoplasm was acidophilic, vacoulated (Plate 3, b) and contained dense populations of PAS and alcian blue-positive secretory granules (Plate 3, c & d). The acid gycoproteins consisted of sulfated and non-sulfated types. Few PAS-positive nonalcianophilic cells were encountered among the principal secretory cells. The nuclei were comparatively small, oval, darkly stained and found close to the

basal lamina. A large sinus-like structure was found occupying mostly the center of each lobule. The sinuses were lined with small cuboidal cells with spherical dark central nuclei and PAS-positive nonalcianophilic cytoplasm.

In electron micrographs, the cytoplasm of the principal cells exhibited a considerable number of membrane-bound secretory vesicles. They were differentiated into two main types. The first type appeared as electron dense spheroidal granules distributed mainly within the apical cytoplasm (Fig. 3, 4). The granules of the second type were considerably smaller, less-electron dense and were encountered within the different cytoplasmic regions (Fig. 3). The protein synthesis machinery (rER) was also encountered.

Seminal vesicles

The tubulo-alveolar end-pieces and the ducts of the goat's vesicular glands were organized into many lobules separated from each other by fibrous septa containing variable amounts of smooth muscle. The epithelial lining of the tubules was identified as simple cuboidal to columnar cells (Plate 4, a). PAS-positive material was observed in most of the epithelial cells indicating its glycoproteineous nature (Plate 4, b). PAS-alcian blue staining revealed the presence of a mixture of acid and neutral glycoproteins. Alcian blue staining at different hydrogen ion concentrations showed that the acidic glycoprotein was of the weakly sulphated type. The nuclei of the secretory cells were oval, lightly stained and located onto the basal lamina. A comparatively large sinus-like structure was commonly noticed occupying the center of each lobule. The sinus lining cells were cuboidal to columnar with PAS-positive nonalcianophilic cytoplasm. Their small spherical or oval nuclei were centrally located.

In electron micrographs, the nuclei were spherical indented and euchromatic. The cytoplasm was less-electron dense and contains elements of rER and a number of electron-dense spherical granules (Fig. 5).

Ampullary glands

The ampullary glands of goats were represented by a dense population of tubulo-alveolar secretory end-pieces located within the propria-submucosa of the terminal portion of the ductus deferens (Plate 5, a). The simple and/or pseudo-stratified columnar cells of the lamina epithelialis were formed of two main cell types: principal columnar cells and basal cells (Plate 5, b). The columnar cells had lightly stained acidophilic, PAS-positive and nonalcianophilic cytoplasm (Plate 5, b). The oval and lightly stained nuclei were located basally. The basal cells posses small, spherical and lightly stained nuclei which were mostly encountered close to the basal lamina. The secretory cells in general, contained a variable number of PAS- positive secretory granules in their upper two thirds. The granules were found to be nonalcianophilic (plate 5, c). The comparatively large,

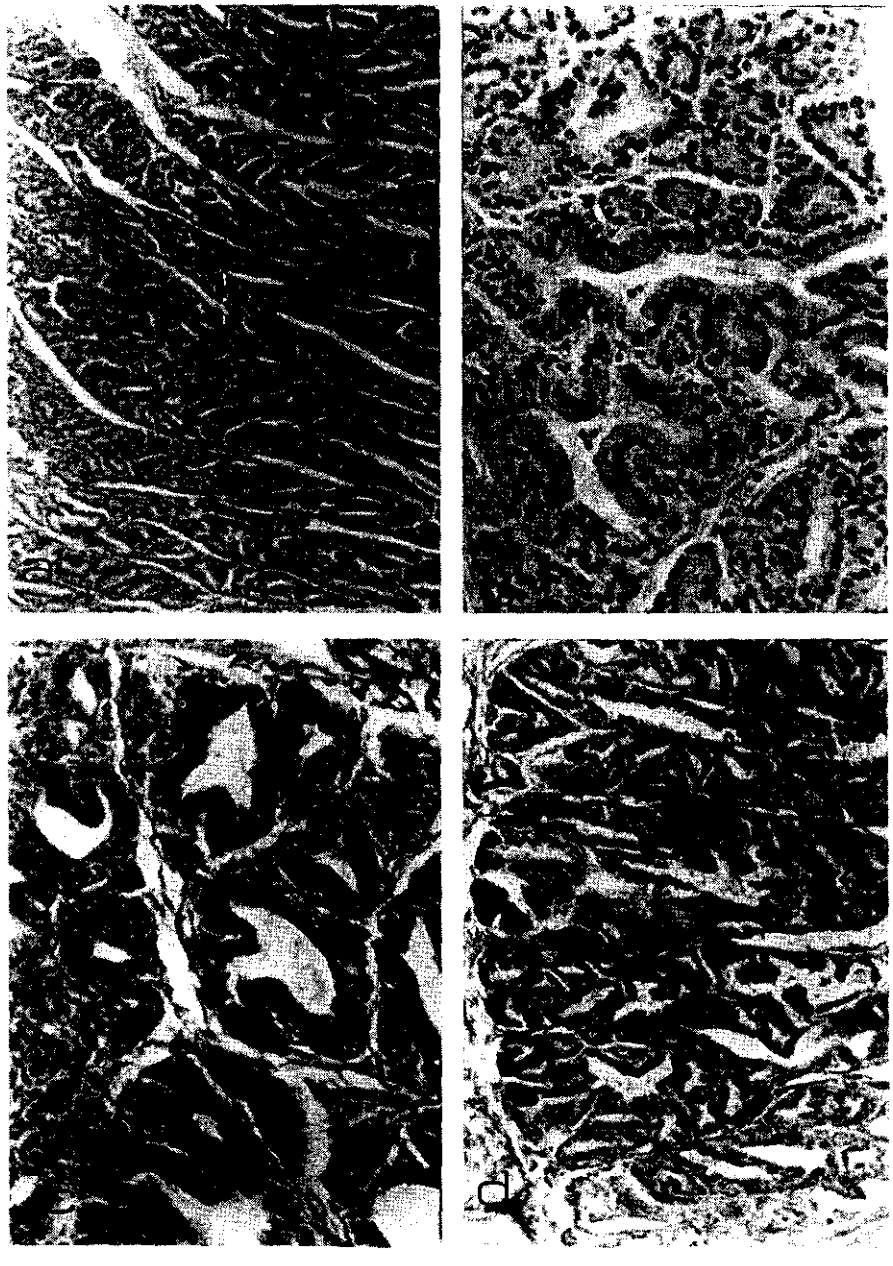

Plate (1): a. Goat's prostrate gland showing the tubulo-alveolar secretory units with wide lumina. H & E, X 100

b. Goat's prostate gland showing that the tubular and alveolar cells posses an acidophilic cytoplasm. H & E, X 400

c. Goat's prostrate gland showing PAS-positive reaction in the cytoplasm of the secretory cells. PAS, X 400

d. Goat's prostate gland showing alcian blue positive reaction in the cytoplasm of the secretory cells. Alcian blue-Van Gieson, X 250.

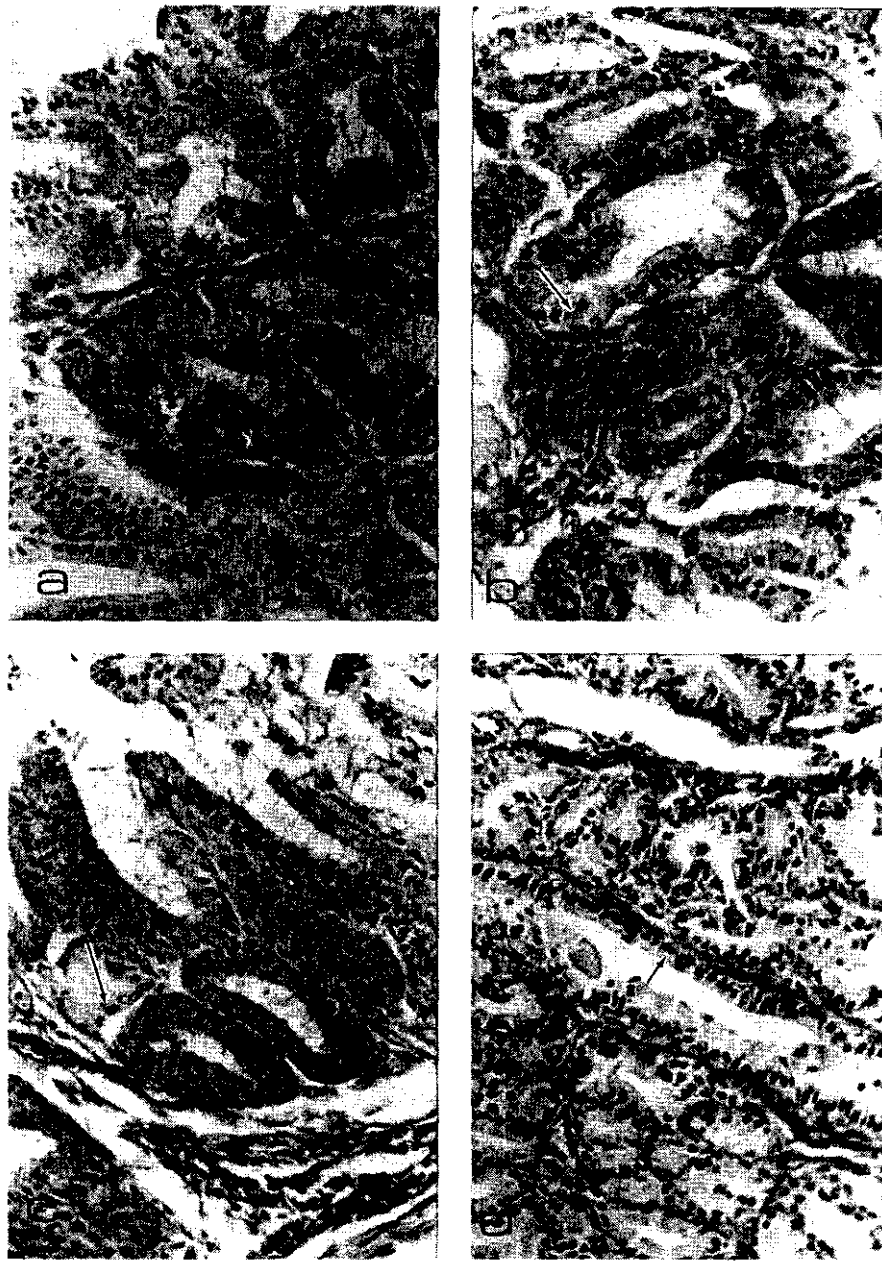

Plate (2): a.Goat's prostrate gland showing small spherical or oval darkly stained and basal nuclei. Alcian blue-Van Gieson, X 400.

b. Goat's prostrate gland showing small spherical or oval darkly stained and basal nuclei. Alcian blue-Van Gieson, X 400.

c. Goat's prostrate gland showing the nonalcianophilic cytoplasm of some of its secretory cells (arrow). Alcian blue-Van Gieson, X 400.

d. Goat's prostrate gland showing the columnar cells lining the interlobular duct (arrows). Van Gieson, X 250.

Fig. (1): Electron micrograph of the goat prostate showing columnar secretory cells exhibiting oval to elongated indented basal euchromatic nuclei with sparse peripheral heterochromatin (N). Note also other organelles such as rER (E), Golgi (G) and few dense granules (arrow). X 2800.

Fig. (2): Electron micrograph of the goat prostate showing well-developed elements of rER (E) and lysosomal-like dense body (L) within the cytoplam of the columnar secretory cells. X 8000.

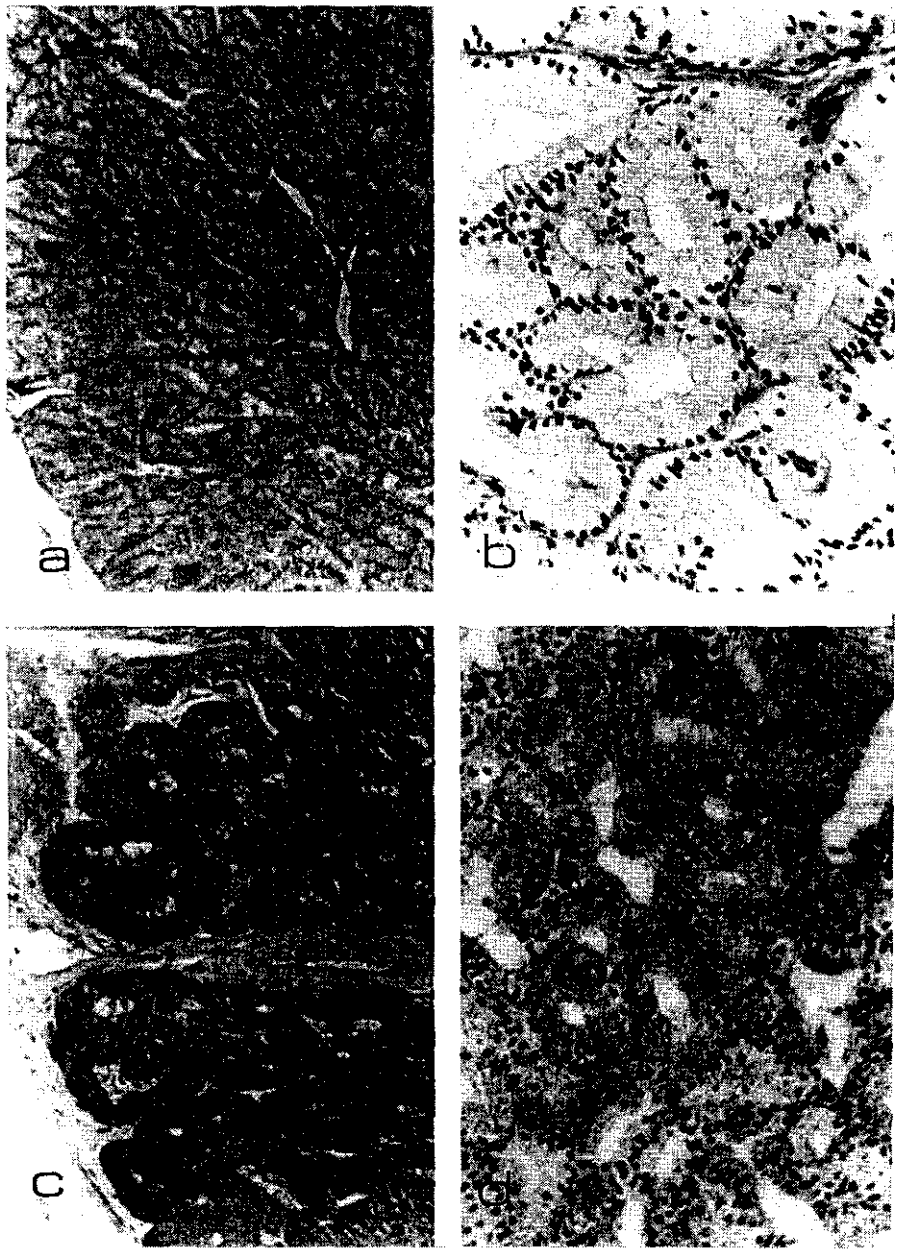

Plate (3): a. Goat's bulbo-urethral gland showing the lobular architecture of the gland and the tubulo-alveolar structure of the secretory units. Alcian blue-Van Gieson, X 100.

b. Goat's bulbo-urethral gland showing its secretory units with wide lumina and vacuolated cytoplasm. H & E, X 400

c. Goat's bulbo-urethral gland showing the PAS-positive reaction of secretory cells. PAS, X 250

d. Goat's bulbo-urethral gland showing the alcian blue positive secretory cells. Alcian blue-Van Gieson, X 400

Fig. (3) : Electron micrograph of the goat's bulbo-urethral gland showing the presence of small (c) and large (d) dense granules within the cytoplam of the columnar secretory cells. X 2800.

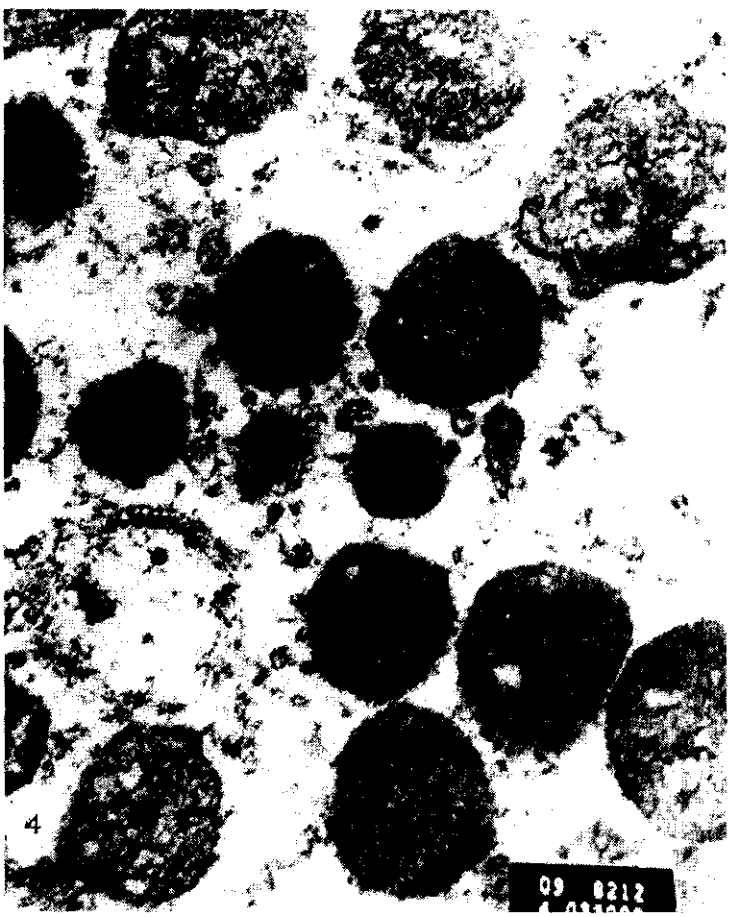

Fig. (4): Higher magnification for the large dense spheroidal granules within the apical cytoplasm of the columnar secretory cells of the goat bulbo-urethral gland. X 22000.

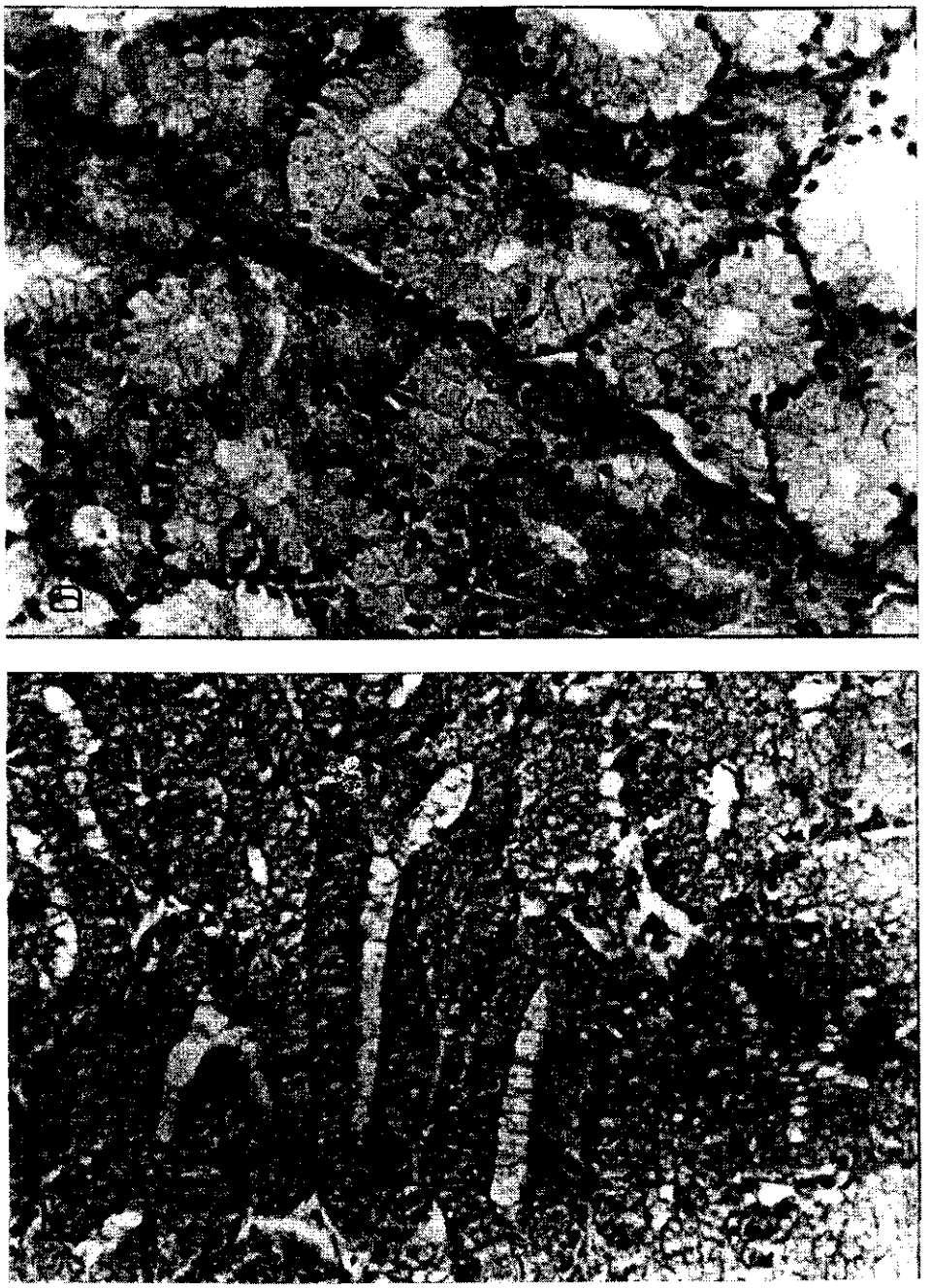

Plate (4): a. Goat's seminal vesicle showing the lobular nature of the gland and the cuboidal to columnar secretory cells that line the adenomeres. (H & E, X 250

b. Goat's seminal vesicle showing PAS-positive cytoplasm of the secretory cells. PAS, X 400

Fig.(5): Goat's seminal vesicle showing the indented spherical euchromatic nucleus (N). Note also the presence of rER (R) and electron-dense spherical granules (G) within the cytoplasm of its secretory cells. X 10000.

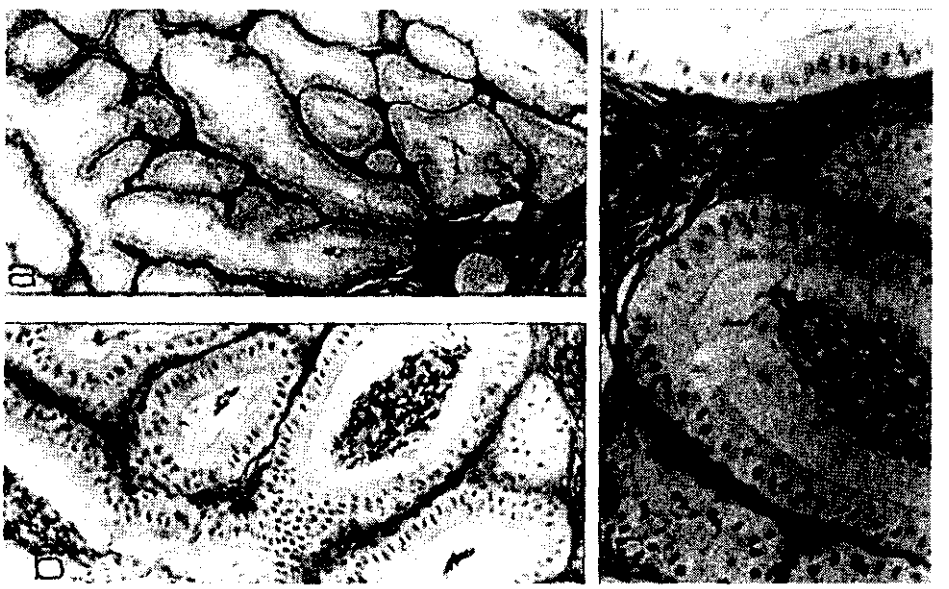

Plate (5): a. Goat's ampullary gland showing the tubulo-alveolar secretory end-pieces. Alcian blue-Van Gieson, X 400

b. Goat's ampullary gland showing the simple and/or pseudostratified columnar lamina epithelialis. Alcian blue-Van Gieson, X 250

c: Goat's ampullary gland showing the nonalcianophilic cells and large number of spermatozoa within one of the secretory end-pieces. Alcian blue-Van Gieson, X 400

Fig. (6): Goat's ampullary gland showing the indented spherical euchromatic nucleui (N). Note also the presence of rER (E), and one of the electron dense spherical granule (S) within the cytoplasm of the cells. X 10000.

ovoid and lightly stained nuclei were located centrally. Unlike the other accessory genital glands, collecting sinuses are absent and the secretory tubules of the ampullary glands joined the surface epithelium of the ductus deferens directly via short ducts. A considerable number of spermatozoa was observed within the lumina of some of the secretory tubules (Plate 5, b & c).

In electron micrographs, the cytoplasm of the principal columnar cells exhibited a well-developed rER and a number of electron dense granules (Fig. 6).

DISCUSSION

Two histochemically distinct types of cells are found to line the secretory tubules of the Baladi goat prostate: principal pyramidal cells with PAS-positive alcianophilic cytoplasm and fewer columnar cells having PAS-positive but nonalcianophilic cytoplasm. Similar findings were observed in rats (Tsukise and Yamada, 1981) and squirrels (Siwela and Tam, 1984). The two cellular forms were either two functional phases of a single cell type or two distinct populations of secretory cells. The secretory cells of the goat prostate glands, therefore differ from those of rats (Wahlqvist et al., 1996) and man (El-Alfy et al.,

2000) where only one single type of secretory cells was described. The present findings were also in a partial agreement with the results of Tsukise and Yamada (1987) in goats who demonstrated the presence of three types of cells containing varying amounts of acid glycoconjugates: mucus-producing cells, protein-secreting cells and cells intermediate between them.

Shackleford and Wilborn (1968) have classified the secretory cells depending on their content of neutral and acid mucopolysaccharides. The authors stated that if secretory cells lack alcian blue reactivity but show PAS reactivity, they should be labeled as serous. On the other hand, if the cells show a positive reactivity for both alcian blue and PAS stains, they should be considered as mucous secreting cells. Based on the aforementioned criteria, we have been found that the goat's prostatic tubules were considered to be of a mixed type since they contain both varieties of cells. The greater abundance of acid mucosubstances that had been demonstrated in the present study lead us to suggest that the prostate glands of goat is mixed, mainly mucous. These findings coincided to a great extent with the findings of Todhunter and Gemmell (1997) who demonstrated that the major bulk of the prostate product is acid mucopolysaccharides. Kanwar and Sheikher (1977) also reported that the columnar epithelium of the prostatic tubules produces a product which is rich in proteins and acid mucopolysaccharides but devoid of lipids. In (1981), Barbour however, found that the major secretory product of the prostate of the wombat is glycogen. A small quantity of acid mucin is produced.

The exact functions of the serous cells in the goat's prostate were still unclear. Sirigu et al., 1995 demonstrated an intense positive immunoreaction for secretory IgA in the serous cells and postulated that the cells might be involved in the transport of immunoglobulins into prostatic secretions.

The morphological pecularities and the absence of bleb-like protrusions from the lumial borders of secretory cells of the goat's prostate might indicate a merocrine secretory mechanism. In 1996, Wahlqvist et al. reported in rat that the secretory mechanism is merocrine in the ventral and lateral prostate, and was mainly apocrine in the dorsal prostate.

The principal cells of the bulbourethral glands were found to be filled with PAS-positive alcianophilic secretory granules. The existence of some cells containing PAS-positive but not alcianophilic granules was also encountered. These findings indicated that most of the gland cells contained variable amounts of acid glycoproteins while the remaining cells contained neutral glycoproteins

only. Furthermore, it has been found that the former cells contained, at least, two types of glycoproteins; sulfated and nonsulfated. Neutral and acid mucosubstances were also detected in the cytoplasm of the secretory and excretory cells of the glands of man (Sirigu et al., 1993), goat (Tsukise and Yamada, 1987) and rats (Tsukise et al., 1979). Also in rats, Nielsen (1976) added that the acid mucosubstances consist mainly of sulfated acid mucosubstances and to a lesser extent of carboxylated acid mucosubstances (sialomucin). More (1991) reported that the bulbourethral glands of calves were divided into two zones according to their secretory products.

Several functions have been attributed to the mucous cells of the bulbourethral glands. One of these functions is the secretion of some blood group antibodies into the seminal plasma. This suggestion is given when it has been found that the mucous cells of the human bulbourethral glands react positively with the blood group antigens (Sirigu et al., 1993). This suggestion agrees with the existence of rER in the secretory cells, as it was observed in the present study. Hellgren et al. (1982) clarified that the secretory granules of the mucous cells of the human bulbourethral glands are found in surrounding capillaries, which suggests that this gland also have an endocrine function.

The epithelium lining the seminal vesicular tubules of the goat were found to contain PAS-positive material in most of the epithelial cells, indicating its glycoprotein nature. Alcian blue/ PAS staining also showed the presence of a mixture of acid and neutral glycoproteins. Alcian blue staining at different hydrogen ion concentrations (1.0 & 2.5) revealed that the acid glycoproteins were of the weakly sulphated type. The glycoprotein nature of the seminal vesicle secretion was previously demonstrated in the glands of other animal species such as rats (Tsukise and Yamada, 1981); goats (Tsukise and Yamada, 1984); and human (Arenas et al., 2001). In guinea pigs, Chan and Wong (1991), however, demonstrated little or no neutral carbohydrates in the apical cytoplasm of the vesicular epithelial cells.

The morphological and histochemical features of the secretory cells that line the seminal vesicle tubules might suggest that the cells secrete their content of granules by the merocrine mechanism, a finding which coincided with those of Wahlqvist et al. (1996) who reported that the cells of the seminal vesicle were covered by long microvilli and suggested that the secretory mechanism was merocrine.

Several functions have been attributed to the glycoproteins secreted by the seminal vesicles. Aumuller and Scheit (1987) stated that the secretion

is rich with seminal antimicrobial protein (SAP). In 1993, Killian et al. detected four types of proteins in the Holstein bull seminal plasma that were correlated with fertility. Osteopontin, a protein that prevail in higher-fertility males was determined to be a highly acidic glycoprotein (Cancel et al., 1997). The above mentioned data seem to support and to explain the presence of rER in the cells of seminal vesicles of male goats.

The secretory tubules of the ampullary glands of the Baladi goats were lined by simple and/or pseudostratified columnar epithelium. It has been found that the later type was formed of two main cell types: columnar cells and basal cells. The principal columnar cells had PAS-positive nonalcianophilic cytoplasm indicating their neutral glycoprotein nature. The neutral glycoprotein nature of the secretory products was previously demonstrated in the glands of human (Riva et al., 1990); salamander (Zalisko and Larsen, 1988), and rabbits (Murakami et al., 1985). The glycoprotein nature of secretion was concomitant with the fine structure of the goat gland cells which contain rER. Zalisko and Larsen (1988) stated that the neutral glycoproteins secreted by the ampullary gland play crucial roles in sperm maintenance.

In the literature, it has been found that rabbits (Kunkelmann and Kuhnel, 1984) and rats (Andonian and Hermo, 1999) demonstrated the presence of bleb-like protrusions on the apical surface of the columnar principal cells suggesting an apocrine type of secretion. Goats seemed to be different from rabbits and rats. The cells were characterized by the absence of such blebs indicating that the cells elaborate their content of granules by a mode other than the apocrine. Such a point, of course, requires further investigations.

A considerable number of spermatozoa wse noticed in the lumina of some of the secretory tubules of the ampullary glands of Baladi goats. A similar finding was demonstrated in the human gland tubules by Riva et al. (1982). The author reported that the lumena of the ampullay glands contain masses of amorphous secretions and some spermatozoa. In rabbits, Kunkelmann and Kuhnel (1984) elucidated that the ampulla ductus deferens was filled with spermatozoa only at sexual rest and was usually empty after ejaculation.

The precise significance for the presence of spermatozoa in the lumen of the ampullary gland tubules remains to be clarified. It has been reported that epithelial spermiophagy seems to be a common event in the vas deferens of mammals (Aumuller et al., 1980; Murakami et al., 1985; Murakami et al., 1982). Piomboni (1997) reported that the epithelial cells in the ampullary region of the vas deferens are involved in the development and functional maturation of the epididymal spermatozoa. Andonian and Hermo (1999) clarified that the epithelium of the vas deferens plays crucial roles in sperm concentration mainly through synthesis and secretion of steroids and elimination of water.

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