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ELECTRON MICROSCOPICAL OBSERVATIONS ON THE ACTIVE VESICULAR GLAND OF BUFFALO

(With 9 Figures)

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مشاهدات بالميكروسكوب الالكتروني على الغدة الحويصلية النشطة في الجاموس

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يتركب النسيج الطلائي الحويصلية المنوية في الجاموس من ثلاثة انواع من الخلايا : خلايا اساسية وخلايا داكنة وخلايا قاعدية تمر الخلايا الاساسية بمراحل دورية مختلفة من الانشطة الفسيولوجية وشوهد بهذة الخلياثلاثة انواع من النتؤات السيتوبلازمية التى تتصل بالجزء العلوى للسيتوبلازم او تطلق حره في تجويف الحويصلات المفرزه . يتباين ميل السيتوبلازم بالخلايا الداكنة لحمض الأوزميك . تشابه سيتوبلازم الخلايا القاعدية مع مثيله بالخلايا الغير مميزه ، ولوحظ احتوائه على كميات قليلة من الجسيمات الخلوية .

SUMMARY

The fine structural examination of the glandular epithelium of the vesicular gland in buffalo-bull revealed three types of cells lining the end-pieces: principal cells, dark cells and basal cells. The principal cells are present in different cyclic stages of physiological activities. Three types of the apical cytoplasmic protrusions were differentiated according to their contents. They appeared either in connection with the apical cytoplasm or found freely in the lumen of the end-pieces. The cytoplasmic matrix of the dark cells exhibited a variable degree of strong osmiophilic affinity and appeared darker than

Table (1) : Showing the least square means ± standard errors of the frequencies (%) of the MBC-senstive stages of the seminiferous epithelia of rat.

Treatments	VII		XII-XIII		XIV	
	Normal without sloughing	Sloughed	Normal without sloughing	Sloughed	Normal without sloughing	Sloughed
Corn oil only (Control animal)	21.17±0.68^{a}	00.00±0.0	10.33±0.36^{a}	00.00±0.00	6.16±0.35^{a}	00.00±0.0
200 mg MBC/ Kg b.w.	9.00 ±0.36^{c}	11.20±0.43	00.00±0.00^{c}	22.31±0.45	00.0±0.00^{c}	6.96 ±0.48
100 mg MBC/ Kg b.w.	11.67±0.7 ^{d}	10.80±0.39	00.0 ±0.0 ^{c}	24.20±37	00.0±0.00^{c}	6.38 ±0.41
50 mg MBC/ Kg b.w.	8.80±0.33^{d}	12.30±0.37	7.33 ±0.35^{b}	3.7 ±0.47	3.33±0.36^{b}	3.0 ±0.31
25 mg MBC/ Kg b.w.	18.83±0.34^{b}	3.30±0.36	10.67±0.32^{a}	00.0 ±0.0	6.67±0.39^{a}	0.0 ±0.0
10 mg MBC/ Kg b.w.	21.37±0.35^{a}	0.00±0.00	10.67±0.37^{a}	00.0 ±0.0	6.27±0.38^{a}	0.0 ±0.0

Different letters mean significant changes.

the other cells. The cytoplasm of the basal cells is simillar to that of the ill-differentiated cells. It contains a small amount of cell-organelles. The axonal terminals in the periglandular connective tissue were also demonstrated.

INTRODUCTION

In ruminants, the seminal vesicle is one of the accessory genital glands which contributes relatively a considerable amount of secretion to the seminal fluid. The component of the seminal vesicle secretion can influence the motility, metabolism and the acrosomal reaction of the spermatozoa (INSKEEP et al., 1985 and AGRAWAL & VANHA-PERTTULA, 1987).

Several studies have been published not only at the light microscopical level but also by using the histochemical and the electron microscopical methods on the seminal vesicles in small and large ruminant (AITKEN, 1959; MOSIMANN, 1959; HAY et al., 1961; KUNZEL & TANYOLAC, 1968a & 1968b; KAINER et al., 1969; KUNZEL et al., 1970; RAMA et al., 1971; SELIM, 1974; BAYOMY, 1976; WROBEL & INCZEDY-MARCSEK, 1977 and AMSELGRUBER & FEDER, 1986). However, the available limited information about seminal vesicle of buffalo-bull have been obtained after using the macroscopical and light microscopical techniques (OSMAN, 1965; FAHMY & OSMAN, 1972; EISSA, 1980 and MOUSSA et al., 1983).

Therefore, the present investigation was undertaken to study the electron microscopy of the secretory end-pieces of the active vesicular gland in water buffalo.

MATERIAL and METHODS

Vesicular glands were obtained from 12 male water buffaloes (age ca. 2 years) immediately after slaughter. These vesicular glands with the pelvic urethra together with their undamaged surroundings were removed. The local supplying arteries (Aa. prosstatica) were canulated, and a rinsing fluid (for composition see WROBEL et al., 1978, shortly; contains PVP, Nacl, Novacain and Thrombophob) was injected after rinsing, perfusion fixation at room temperature was performed with the formaldehydeglutaraldehyde fixative as described by KARNOVSKY (1965). Tissue blocks representing the different regions of the vesicular gland were removed for examination. Small pieces

were cut and washed in 0.2M cacodylate buffer. After osmication (1% OSO4) the material was dehydrated in graded ethanol and embedded in ERL 4206 (SPURR, 1969). Semithin sections were stained with methylene blue-azur II after RICHARDSON et al (1960). Ultrathin sections were mounted on copper grids, stained with uranyl acetate and lead citrate (REYNOLDS, 1963) and examined with a Zeiss EM 10 electron microscope.

RESULTS

Semithin sections of the vesicular glands revealed oval or round-shaped sections of the glandular end-pieces building the parenchymal tissue. They were of various sizes and supported by a well-developed interstitial connective tissue containing abundant blood capillaries. The lining epithelium of the glandular end-pieces (Fig. 1 & 2) composed of tall-columnar principal cells and basal cells resting upon a well-distinct basal lamina. The principal cells exhibited hexagonal shaped structures in cross sections (Fig. 2). Some of these principal cells have been strongly stained after using methylene blue-azur II (Fig. 1 & 2). They were distinguished by their darkely stained cytoplasm. The basal cells (Fig. 2) were round, oval or flat in shape with centerally located nuclei. They were mostly distributed singly. Although the height of the glandular epithelium varied from one alveolar end-pieces to another, it remained approximatly equal in height within the same end-piece. The principal cells contained distinct apical fine granules. The nuclei of the principal cells were located in the basal portions and appeared oval in shape. In the majority of the end-pieces, the apical portions of the principal cell were pulged into the lumen forming round-shaped protrusions of various sizes. These cytoplasmic protrusions were found freely in the lumen of the end-pieces (Fig. 1). This might indicate the presence of an apocrine mode of secretion.

The electron microscopical examination of the vesicular gland epithelium demonstrated that, the tall-columnar principal cells were rich in cell organelles and carrying apical cytoplasmic protrusions in different developmental phases (Fig. 4 & 7). In some less active end-pieces, the glandular cells were columnar in shape and contained relatively few cell organelles and showing ill-prominent cytoplasmic protrusions (Fig. 8).

In the active principal cells, the basal portions were occupied by slightly elongated nuclei (Fig. 4). They were characterized by their irregular deep invaginations of the nuclear membranes or indentations. The

heterochromatin tend to assume either a marginal positon, condensed along the inner nuclear membrane; or few coarse flakes and fine granules scattering in karyoplasm. Parallel and flattened cisternal lamellae of granulated endoplasmic reticulum were located in the infranuclear area and arranged in semi-concentric manner (Fig. 3). They frequently embraced a spherical lipid globule possessing a limiting membrane. Zone of intercisternal transport (Fig. 3) were demonstrated, where connections between the adjacent cisternae of ER were found. In the supranuclear region, well-developed Golgi-complex (Fig. 4) in the form of triangular shaped structure was frequently encountered. Small vesicles and vacuoles of different size were observed in close association with the Golgi-membranes. In the perinuclear zone, parallel and flattened lamellae of rough endoplasmic reticulum were frequently found.

The middle portion of the principal cells was generally occupied by endoplasmic reticulum that showed frequently a considerable degree of variation. In some cells, the endoplasmic reticulum appeared in the form of closely packed, small tubules or vesiculated structures. In between them, few ribosomes and vesicles containing eccentric dense core were distributed (Fig. 7). While in other cells, flattened parallel cisternal lamellae of rough endoplasmic reticulum were arranged parallel to the longitudinal axis of the cell (Fig. 4 & 5). In addition, group of mitochondria of cristae type were observed. They appeared sometimes vesicular in shape. The mitochondrial matrix was of moderate density, and occasionally, matrix granules were found. Mitochondria were found anywhere but tended to be concentrated along the cell membrane.

The apical cell-portions (Fig. 5 & 7) revealed mostly irregular apical free borders. In areas free of cytoplasmic protrusions, the apical free surfaces carried a small number of finger-shaped fine microvilli containiing electron-dense cores. According to the contents of the apical cytoplasmic protrusions, they were differentiated into three types. The first type (Fig. 4) contained small and short isolated cisternae of rough ER. These cisternae were vesicular, and appeared rounded or elliptical in shape. They were filled with homogenous material of moderate electron density. The content of the second type (Fig. 4) was composed of agranular small vesicles and large vacuoles of different forms, as well as short tubules of variable shape containing non-electron dense material. Their surrouding matrix appeared homogenous electron dense. This second type of cytoplasmic protrusions was encountered at the apical portions of the dark cells. The third type of the cytoplasmic protrusions (Fig. 7) was characterized by the occurrence of a

well-developed smooth membranous structure. It was composed of very fine tubules of variable shape and vesicles. They were numerous and crowded, and contained nonelectron dense material.

In the apical cytoplasm (Fig. 5 & 7) many electron dense bodies of different forms and size were distinguished, which may be of a lysosomal nature. Also, flattened, parallel lamellae of partially granulated ER were arranged in circular form. They were frequently seen surrounding a homogenous electron dense body. Secretory vesicles of various sizes were usually found (Fig. 7). Each one consisted of a minute electrone-dense granule, sometimes with irregular outline, occupying an eccentric position inside a vacuole. The interval between the minute granule and the vacuolar wall varied greatly. The dark cells could be also differentiated by the electron microscope. Their cytoplasmic matrix exhibited mostly a variable degree of strong osmiophilic affinity, and appered darker than other principal cells (Fig. 4).

On the apicolateral borders (Fig. 5), the plasma membranes of the adjacent principal cells were attached together with well developed typical juncional complexes (zonula occuludens and zonula adherens). Few cytoplasmic processes were interdigitated together. Many small desmosomes were demonstrated along the smooth lateral border (Fig. 4 & 7). On the lateral border of the cell, mitochondria in close association with desmosome to form mitochondria-desmosome-complexes were oftenly seen (Fig. 7). Basally, the plasma membrane appeared smooth and attached with the basal lamina by hemidesmosomes.

The electron microscopical pictures of the basal cells demonstrated that the cytoplasmic matrix was lacking in cell organoids (Fig. 6). Very few short profiles of rough endoplasmic reticulum, a few amount of free and polyribosomes and small mitochondria have been demonstrated. Many bundles of microfilaments were distributed in the cytoplasm. The nucleus was oval in shape. Its contour displayed deep fissur (Fig. 8 & 9). The heterochromatin were concentrated on the nuclear membrane. They were attached by the principal cells with a small number of small desmosomes and by the basal lamina with hemidesmosomes. In addition, nerve fibers perforating the basal lamina and occupying an intercellular position were observed (Fig. 9). The intraepithelial unmyelinated nerve fibers contained microtubules.

DISCUSSION

The fine microscopicel investigations of the glandular active end-pieces in buffalo showed that, the lining epithelium is consisted of a single layer of columnar or tall-columnar principal cells and small oval basal cells. These observations supports the light microscopical results by FAHMY & OSMAN (1972); EISSA (1988) and MOUSSA et al. (1983).

The active principal cells appeared columnar or tall- columnar carrying cytoplasmic protrusions. In addition, their content of cytoplasmic organlles varied slightly from one end-piece to enother. But the height of the principal cells remained approximatly equal within the same end-piece. These observations suggest that the end-pieces undergo different stages of cyclic physiological activities. Also, all glandular cells within. The same end-Piece probably lie under the same influence of a certain secretory factor.

Dark cells scattering in between glandular cells of buffalo seminal gland have been also mentioned in bovine seminal vesicles by MANN et al. (1949); EGLI (1956); CONS (1957) and AMSELGRUBER & FEDER (1986). In fact their function is still under consideration, and variously interpertated. MANN and his co-workers (1949) have suggested that they represent a specific cell lineage, with the possible function of transmitting fat from the basal cell to the lumen. Another interpretation has been presented by EGLI (1956) and CONS (1957); these dark cells merely represent glandular cells in stages of cyclic undergone by all principal cells, and they are exhausted cells at the late stage of activity. After a recent fine investigations in the cell and cytoplasmic matrix by GHADIALLY (1982), and after using immersion- and perfusion-fixaion by AMSELGRUBER and FEDER (1986), they supposed that the darkness of their cytoplasm, in fact, may be due to a limited changes after fixation as a result of osmostianbility of the cell, particularly, the dark cells increase in number after immersion-fixation (AMSELGRUBER and FEDER, 1986).

In regard to the apical cytoplasmic protrusions, our observations demonstrate 3 types of cytoplasmic protrusions. This indicates that the sceretory process in buffalo takes place approximatly through the mechanism of apocrine sceretion, particularly, the cytoplasmic protrusions are observed still in connection with the glandular cells and also free in the lumen. The presence of 3 types of apical cytoplasmic protrusions of different contents reflects perhaps that various substances are synthetized in the cytoplasmic protrusions. This may also suggests that the glandular end-pieces of the

buffalo vesicular gland differ from each other at least partially in their secretion.

The results of this investigation demonstrated clearly that, the basal cells of the vesicular gland in buffalo were poorly differentiated. They contained small amount of cell organelles. About the physiological role of the basal cell, there are different interpretations. The basal cells in the bovine seminal vesicle are charcterized by accumulation of lipid globules in the cytoplasm. Therefore, many authors considered that, the basal cells may be responsible for the storage and secretion of lipid into the blood circulation (WROBEL & INCZEDY-MARCSEK, 1976 and AMSELGRUBER & FEDER, 1986). Moreover, they may also share in synthesizing prostaglandins (MANN & LUTWAK-MANN, 1981). But, due to the lipid in the basal cell of buffalo vesicular gland do not actually represent a distinct constituent, our conception about the basal cell is limited in its supporting and regenerating function.

Our electron microscopical observations concerning the periglandular nerve terminals stated that, many unmyelinated nerve fibers and axonal terminals, which are partially devoid of perineural sheath, are located just under the basal lamina of the seminal gland epithelium. A recent ultrastructural study by ABOU-ELMAGD & WROBEL (1989), decomented that the nerve fibers penetrate the basal lamina and form intraepithelial neuroglandular synapses in buffalo prostatic epithelium. Therefore, a similar neuroglandular synapse in the other accessory sex glands of buffalo may become acceptable as a common morphological feature. The same results are also recorded in the guinea-pig seminal vesicle (AL-ZUHAIR et al., 1975).

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LEGENDS

Fig. 1: The glandular epithelium of the end-piece. Apical cytoplasmic protrusions (arrow) and darkely stained cells (arrowhead) are easily identified. Semithin section stained by methylene blue-Azur II. x 780.

Fig. 2: The glandular epithelium of the vesicular and consists of principal cells (asterisk), darkely stained cells (arrow) and basal cells (arrowhead). Semithin section stained by methylene blue-Azur II. x 780.

Fig. 3: Infranuclear region of a glandular cell. Circular arranged flat cisternae of rough ER (arrowheads) around fat globules (F). Zone of intercisternal transport (arrow). x29200.

Fig. 4: Principal cells with basal nuclei. Notice the first type (I) and the second type (II) of apical cytoplasmic protrusions. The middle cell has darkely stained cytoplasm. Golgi-complex (G), apical junctional complex (arrow). Small desomosomes (arrowheads), mitochondria (M). x 7300.

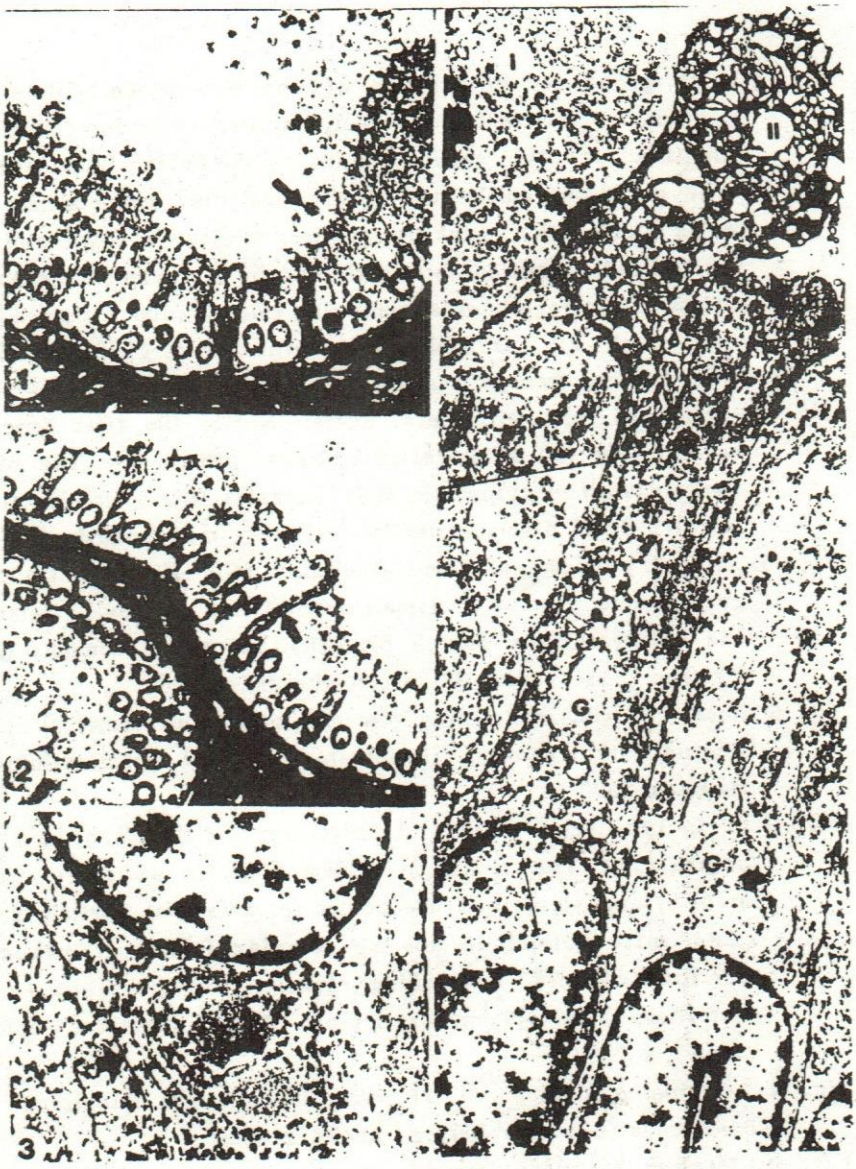
Fig. 5: Apical portions of some glandular cells. Notice early formation of cytoplasmic protrusion (small arrow) and microvilli (arrowheads) on the apical surface. Rough ER (ER), junctional complex (large arrow), electron dense bodies (large arrowheads). x 7300.

Fig. 6: Basal cell (BC) containing few cell organoids. Principal cell (PC), basal lamina (arrowheads), fibroblast (F) in the periglandular connective tissue. x 7300.

Fig. 7: Principal cells with the third type (III) of apical cytoplasmic protrusions. The apical cytoplasm contains secretory vesicles (small arrows) and electron dense bodies (arrowheads). Partialy granulated ER (ER), mitochondriadesmosome-complex (small arrowheads). Middle portion (asterisk) containing closely packed tubules or vesiculated cisternae of endoplasmic reticulum. Apical junctional complex (large arrow). x 7300.

Fig. 8: Principal glandular cell (PC) with less distinct cytoplasmic protrusions. Basal cells (BC) with fissured nuclei x 4000.

Fig. 9: Higher magnification of the basal part of fig 8. Notice the intraepithelial nerve axons without perineural sheath (arrow) in the intercellular space. Basal lamina (arrow heads). x 14600.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