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**THE FINE STRUCTURE OF THE EPITHELIUM
 OF BUFFALO EJACULATORY DUCT**
 (With 3 Figures)

By

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التركيب الدقيق للنسيج طلاشي في القناة القاذفة في الجاموس

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تم فحص القنوات القاذفة في الجاموس بالميكروسكوب الإلكتروني النافذ . تبين
 هذه القنوات بنسيج طلاشي كاذب يتكون من خلايا عمادية أساسية وخلايا قاعدية . ولقد
 وجد أن الخلايا القاعدية قليلة التميز بينما شوهدت الخلايا العمادية الأساسية في أنشطة
 فيسيولوجية مختلفة . ظهرت إحدى أنواع الخلايا العمادية الأساسية مبينا نشاط إمتصاصي
 إنتقالي للسوائل وتميز بوجود عديد من الميتوكوندريا المتمركزة في قاعدة الخلايا . بينما
 ظهر نوع آخر من الخلايا العمادية الأساسية مقحمة في إنتهاء النطفات وكانت هذه الخلايا
 مدعمة بعديد من الفجوات الملتهمة وتحتوي على نطفات موهزمة وأيضاً تحتوي على
 جسيمات حالة بلعية مكثفة .

SUMMARY

The buffalo ejaculatory ducts are examined by transmission electron microscopy. They are lined by pseudostratified columnar epithelium. The lining epithelium is composed of columnar principal cells and basal cells. The basal cells are less differentiated, while the principal columnar cells are observed in different physiological activities. One principal cell-type shows absorption and transport activity and is characterized by many small basally located mitochondria. Other principal cell-type is involved in phagocytosis of spermatozoa and is provided with many phagocytic vacuoles containing digested spermatozoa and condensing phagolysosomes.

INTRODUCTION

In buffalo, as in ruminants, the excretory duct of the seminal vesicle joins the terminal part of the deferent duct, beyond the ampullary portion, to form the short ejaculatory duct which transports the spermatozoa and seminal plasma to the pelvic urethra (KAINER *et al.*, 1969). The ejaculatory ducts, particularly in buffalo have received a little attention by morphologists, although they connect between copulatory organs and that of sperm production. Therefore, data concerning the histology and the ultra-structure of buffalo ejaculatory duct are not available.

More recent studies have shown that a considerable amount of spermatozoa are phagocytosed in ejaculatory duct of bovines (ABOU-ELMAGD and WROBEL, 1990), cats (MURAKAMI *et al.*, 1984), rabbits (MURAKAMI *et al.*, 1985), monkeys (RAMOS, 1972);

ABOU-ELMAGD & KELANY

MURAKAMI *et al.*, 1981) and also man (RIVA *et al.*, 1982; MURAKAMI *et al.*, 1982; COSSU *et al.*, 1983). Furthermore, ABOU-ELMAGD and WROBEL (1990) described a phagocytotic activity in the bovine ejaculatory duct. These observations indicate that the epithelium of the ejaculatory duct may perform special additional functions besides serving as a pathway for spermatozoa. In continuation of our studies on ruminant reproduction, we describe the epithelium of the buffalo ejaculatory duct.

MATERIAL and METHODS

Ejaculatory ducts were obtained 10-20 min. after slaughter. Perfusion fixation of the pelvic urethra through the prostatic arteries was performed. For light microscopical examination, rinsing procedure and perfusion with Bouin's solution were performed as described elsewhere (WROBEL *et al.*, 1978). From paraplast-embedded material 5-7 μ m thick sections were prepared and stained with modified Masson-Goldner trichrome. For transmission electron microscopy, fixation was performed with the formaldehyde-glutaraldehyde fixative as described by KARNOVSKY (1965). Small pieces of the ejaculatory duct were separated and washed in 0.2 M phosphate buffer. After osmication (1% OsO₄) the small blocks were dehydrated in graded ethanol and embedded in ERL 4206 (SPURR, 1969). Semithin sections were cut and stained with Methyleneblau-Azur II (RICHARDSON *et al.*, 1960) for light microscopical examination. Ultrathin sections were mounted on copper grids and stained with uranyl acetate and lead citrate (REYNOLDS, 1963) and examined with Zeiss EM 10 A electron microscope.

RESULTS

The semithin sections of the Buffalo ejaculatory duct (Fig. 1) show that the lumen of ejaculatory duct is irregular in shape and contains invaginations. The lining epithelium is composed of pseudostratified columnar epithelium. The cells were mainly arranged in two layers resting on the basal lamina. These cells are differentiated into two types: Tall-columnar principal cells and small basal cells locating between the basal slender portions of the columnar principal cells. Phagocytosed spermatozoa and free mononuclear cells are demonstrated intraepithelially.

The fine structure of the principal cells (Fig. 2) demonstrates that the apical cytoplasm bulges slightly into the lumen. Laterally, the plasma membranes of the adjacent principal cells run straight, only few simple interdigitations are seen. Apical junctional complexes and a few number of desmosomes connect the cells together. Generally, the nuclei of the principal cells are highly variable in shape. Very often, deeply fissured, irregular nuclei are seen. Clumps of various size, as well as many small and fine particles of chromatin are located on the inside of the nuclear membrane or scattered in the nucleoplasm. Often, the nucleolus is found excentrically. Less active ER are observed. Very thin less electron dense smooth membranes with indistinct lumen are distributed supra- and infranuclearly. Also, many principal cells are highly differentiated and demonstrate features of endocytotic, fluid absorption and transport activities. They possess apical microvilli and slightly stained slender basal portions containing many small mitochondria of variable shape. Other principal cells showing phagocytic activity, particularly

BUFFALO EJACULATORY DUCT

spermiophagy, are observed. They are characterized by smooth apical surface. The cytoplasm contains many portions of spermatozoa lying within phagocytic vacuoles and condensing phagolysosomes. Golgi-apparatus is poorly developed or indistinguishable. A few number of very electron dense apical bodies of lysosomal nature and bundles of microfilaments are seen. Hemidesmosomes connect the base of the cell and the electron dense basal lamina. The oval basal cells (Fig. 3) are characterized by ill-differentiated dense cytoplasm containing few cell-organoids. Their nuclei are highly variable in shape and deeply fissured. In addition, intraepithelial lymphocytes (Fig. 3) are usually observed.

DISCUSSION

In buffalo ejaculatory duct, the lining epithelium is composed of tall columnar principal and small, oval basally located cells. This is morphologically nearly identical to that described in the bovine ejaculatory duct by ABOU-ELMAGD and WROBEL (1990) and in ductus deferens and seminal vesicles (HENDRICH, 1905; CONS, 1957; KAINER *et al.*, 1969; KÜNZEL & TANYOLAC, 1968; WROBEL & MARCSEK, 1976 and AMSELGRUBER & FEDER, 1986). However, the basal cells in bovine ejaculatory duct contain lipid droplets of different sizes, a feature which characterizes the bovine reproductive organs deriving from the wolffian duct.

The morphology of epithelial principal cells reflects, besides their protective function, two forms of physiological activities: Fluid absorption and transport, and spermatozoa phagocytosis.

Cells showing fluid absorption and transport activities are characterized by apical microvilli and many basal mitochondria. ABOU-ELMAGD and WROBEL (1990) observed the similar activity in the bovine ejaculatory duct. The cells contain a system of microvesicles and lysosomes occupying the area under the free surface. They are very necessary for absorption and degradation. Moreover, the basal mitochondria facilitate the basal transport of fluid into the surrounding subepithelial stroma. This activity may be interpreted as a physiological process in order to eliminate the fractions of seminal plasma that normally flow from the ampullary glands and seminal vesicles into the ejaculatory duct.

In bovine bull, the number of spermatozoa obtained by exhaustive ejaculation is less than half of the estimated number of spermatozoa produced by the seminiferous tubules (AMANN and ALMQUIST, 1962). Although the exact mechanisms and functional significance of sperm elimination in the male genital tract is not yet completely understood. ABOU-ELMAGD and WROBEL (1990) reported two possibilities of sperm loss: Voidance in urine and resorption by the epithelia of the excurrent duct system.

The epithelial spermiophagy, that has been already reported in to the seminiferous tubules (DYM, 1974; NYKÄNEN, 1979), rete testis (BURGOS & CAVICCHIA, 1975; HOLSTEIN, 1978; GOYAL, 1982 and SINOWATZ *et al.*, 1979), has been interpreted as a selective removal of degenerated or abnormal spermatozoa. On other hand, the spermiophagy of spermatozoa by epithelium of ejaculatory duct is considered as a different

ABOU-ELMAGD & KELLANY

process. It can clear the lumen from spermatozoa that remain after ejaculation or flow passively from the vas deferens. Spermiophagy in ejaculatory duct is also observed in bovine (ABOU-ELMAGD and WROBEL, 1990) and in man (COSSU *et al.*, 1983).

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BUFFALO ELACULATORY DUCT

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LEGENDS

Fig. 1: Semithin section from the lining epithelium of the buffalo ejaculatory duct showing pseudostratified columnar epithelium; principal cell (P) and basal cell (B) infiltrated by free mononuclear cells.

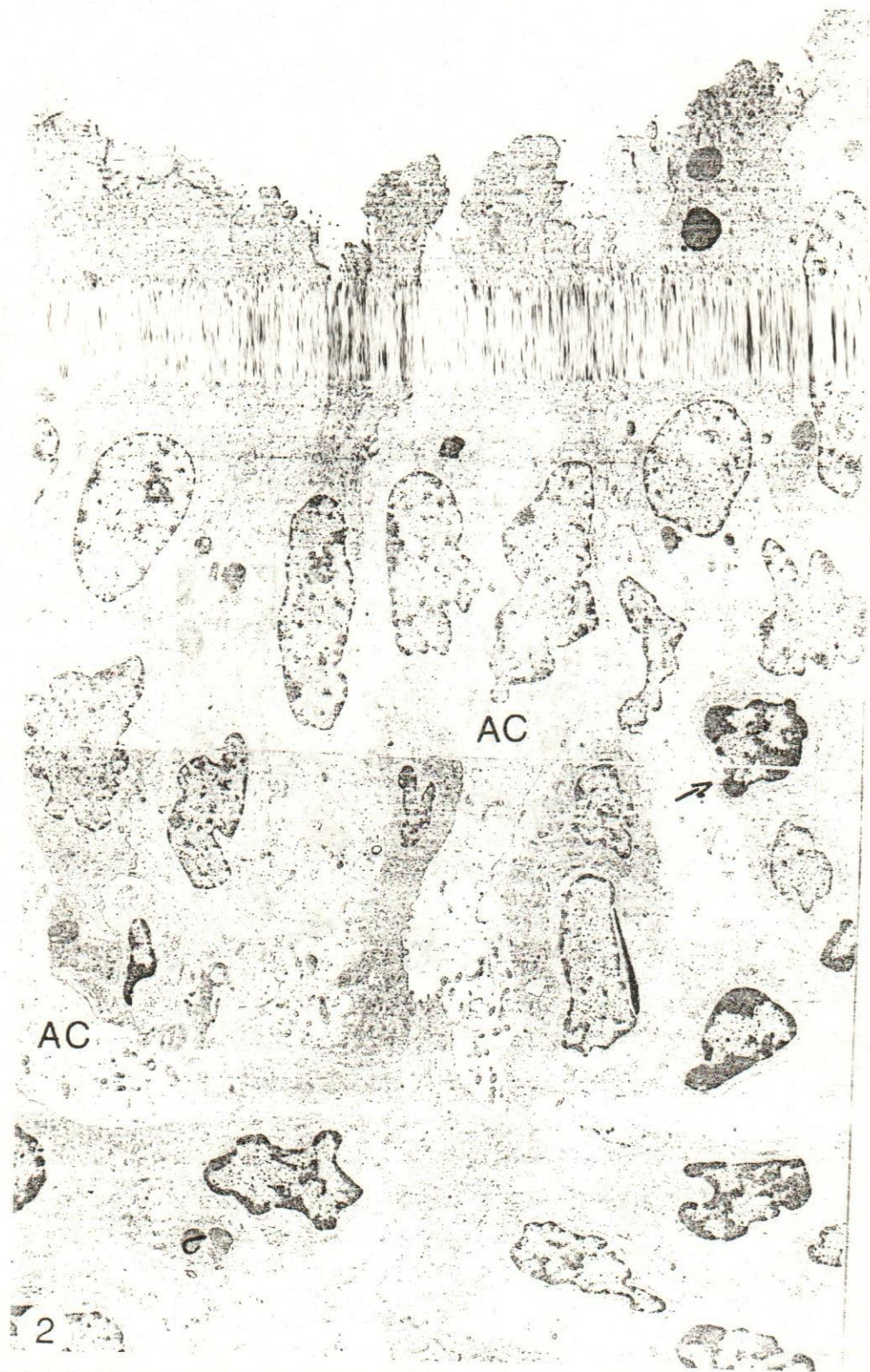
Methylenblau-Azur II x 800.

Fig. 2: Transmission electron micrograph showing the feature of the lining absorptive principal cells (AC), particularly, the basally located mitochondria and apical microvilli, Intraepithelial Lymphocytes (arrow). X 9000.

Fig. 3: Transmission electron micrograph showing spermiphagy in epithelial cells (SC) and basal portion of absorptive principal cell (AC) containing many mitochondria. Basal cells (BC) and intraepithelial Lymphocyte (arrow) can be identified. X 9000.



BUFFALO ELACULATORY DUCT



BUFFALO ELACULATORY DUCT

