THE MICROMORPHOLOGY OF HEPATOID CIRCUMANAL GLAND IN NIGERIAN DOGS

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SUMMARY

The hepatoid circumanal glands (HCGs) were found in dogs of all age group, in both sexes and appeared to be ductless. There were usually two glands separated by a hair follicle. A lobe of a gland was made of 20 lobules or more. Each lobule consisted of polygonal or polyhedral non-vacuolated cells with one or more nucleoli. Ultrastructurally, hepatoid circumanal gland appeared to be involved in both protein and lipid metabolism. The structure as found in our local dogs bare similarity to the HCGs in exotic breeds previously reported. There is need for more studies to be done on the functional morphology of HCGs and their role in Transmissible Venereal Tumour in African dogs.

KEY WORDS: Dogs. Micromorphology. Hepatoid circumanal gland.

INTRODUCTION

The HCGs are regarded as solid, lobulated, glands arranged in cords resembling closely packed liver cells found in a uniform circle extending up to 2.0cm from the anal orifice (Nielsen and Aftosmis, 1964; Isitor, 1983). There is the viewpoint by these authors that HCGs have an abortive nature, lack efferent ducts, and show no signs of secretory activity. However, most modern researchers indicate that these glands are actively functioning structures (Atoji et al., 1998; Shabadash and Zelikina, 2002). Authors have classified canine HCGs as ductless (Petterino et al. 2006; Pisani et al., 2006) and others are of the opinion that HCGs are exocrine glands (Shabadash and Zelikina (2002). Atoji et al. (1998) suggested that HCGs are just circumanal bodies instead of glands. Nonetheless, there is a concensus that HCGs are a leading source of neoplasia (Nielsen and Aftosmis, 1964; Alexander et al., 1976, Bostock, 1986).

The circumanal region has been an area of interest to scientists because of the hepatoid circumanal glands which have been known to be a leading source of neoplasm in Europe and America. The neoplasm from these glands ranks third in frequency in all canine neoplasia after mast cell and mammary tumours (Nielsen and Aftosmis, 1964; Alexander et al., 1976, Bostock, 1986). The importance of canine neoplasia has been pointed out in comparative medical studies because this species share the same environment with man and most of the neoplasia seen in them has been found in man (Kaplan, 1964; Cohen et al., 1974).

All the reports in the literature have been on the circumanal glands of exotic breeds of dogs. There is no study done on the local breeds of dogs. The objective of this work therefore is to study HCGs at both the light microscopic and electron microscopic levels so as to generate information on our local breeds of dogs.

MATERIALS AND METHODS

A total of 10 dogs, comprising 5 healthy puppies and 5 healthy adults of both sexes were utilized for this study. The dogs were divided into two groups A and B, consisting of 5 puppies and 5 adults respectively. Perianal skin tissue were collected from each dog in both groups using single edged razor blades while the animals were anesthetized with short acting barbiturate.

The tissues collected were divided into two: half of the tissues collected were fixed in 10% formol saline which was later processed for light microscopy. The remaining tissues collected were immediately minced into smaller bits and quickly prefixed at room temperature in 2.5% glutaraldehyde solution buffered with 0.1M cacodylate HCL solution (pH 7.4) for a maximum of 3 hours. The specimens in this group were processed for electron microscopy. The tissues were washed in 0.1M cacodylate buffer for 3 consecutive intervals of 10 minutes each. Samples were postfixed in 1% Osmium tetroxide cacodylate buffered solution for a maximum period of 2 hours at room temperature.

The postfixed tissues were washed at five minutes intervals for 30 minutes in cacodylate buffer (pH 7.4). Samples were dehydrated in ascending grades of acetone and infiltrated in freshly prepared Epon-araldite. Samples were left in the desicator for 2 days so that the acetone could evaporate, then embedded in a freshly prepared Epon-araldite in the moulding capsules and incubated in the oven at 30oC for 4 hours, 45oC for 12 hours and 60oC for 2 days.

The embedded tissue was sectioned with a Reichert ultramicrotome. Thin sections (0.5 µm) were stained with toluidine blue and viewed with the light microscope for general orientation of areas in the tissue. Light micrographs were obtained from the thin sections at varying magnification. Ultra thin sections of silver and gold interference were mounted on 300 mesh copper grids, double stained with uranyl acetate and lead citrate

solution and viewed with a Philips EM201 Transmission Electron Microscope. Electron micrographs were obtained at varying magnifications.

RESULTS

The HCGs were located in the dermo-hypodermal junction in the puppies and were located deeper in the corium of the adult dogs where they were limited by striated muscle fibers. The HCGs were elongated in shape and usually paired, with the hair follicle in the centre separating the two glands (Plate 1). They occurred laterally to the hair follicle without any communication between them. The HCGs were observed to be multilobulated and the number of lobules varied per gland and could be up to 20 or more (Plate 5). These lobules were demarcated from each other by connective tissue. The lobules had various shapes ranging from oval, square, rectangular to polygonal. There appeared to be no connection between the gland and the surrounding blood vessels or between the gland and the corresponding hair follicle. The lobes and the lobules of the HCGs were surrounded by rich capillary network and larger blood vessels.

Histologically, HCGs in puppies appeared to be of two types: this observation is based on the location of the vacuolated cells in the lobe of the gland. One type appeared to have sebaceous-like cells in its superficial portion with a deeper portion of compact solid HCG lobules (Plate 2). The second type had few vacuolated sebaceouslike cells in the centre of a lobe, and this was surrounded by compact, non vacuolated glandular cells of HCG lobules. The vacuolated cells observed in this age group (puppies) appeared to diminish as the animal grew older. The HCG in male puppies appeared to be more developed than that of the female puppies. The HCG cells of the adult dog had no vacuolation. The glands appeared bigger and had more lobules to a lobe. Intralobular cyst was observed in the HCG of the adult dog (Plate 4). In the adults, HCGs were extensively developed with cyst formation. The degree of HCG development was higher in the adult male dog than the adult female dog.

Each lobule of the gland comprised of inner,

secretory, compact cells and surrounded by a single layer of peripheral cells (Plates 3 and 5). The inner, secretory, glandular cells were polygonal in shape. They were closely packed with reduced intercellular spaces and had little or no intercellular canaliculi between them. The cytoplasm of the inner, secretory, glandular cells was quite abundant and contained spherical eosinophilic granules. Cellular outline was very distinct. The nucleus of the glandular cell was ovoid with basophilic nuclear envelope. Some cells had one or two nucleoli and scattered chromatin. The inner, glandular, secretory cells appeared to be of two types based on the intensity of the cytoplasmic matrix to H & E or toluidine blue stain. Some of the cells appeared to have light cytoplasm while others had dark cytoplasm. There was no definite pattern to the arrangement of the light to dark cells.

The peripheral, basal cells of the HCG lobule had deeply staining hyperchromatic, large, elongated, squamous nuclei, with scanty cytoplasm (Plates 3 and 5). Cellular outline and cytoplasm were not discernible (Plates 3 and 5). Some tubular structures were observed in the connective tissue adjacent to the HCGs. These tubular structures were lined by two layers of cuboidal epithelium (Plate 3).

Ultrastructurally, the typical cell of the HCG consisted of three portions: the distinct nucleus, the cytoplasm and the plasma membrane. The nucleus was bounded by trilaminar double nuclear envelope thrown into folds. The electronlucent perinuclear space was connected with the cisternae of the endoplasmic reticulum and abundant nuclear pores opened into the cytoplasm through the nuclear envelope. The condensed heterochromatin material was abundant on the nucleoplasmic aspect of the inner nuclear envelope while the eurochromatin material was numerous in the nucleoplasm (Plate 6).

The cytoplasm of the HCG was limited by the plasma membrane or the cell membrane (Plate 7). The cell membrane consisted of three layers: two electrondense inner and outer layers separated by electronlucent layer. The organelles present in the cytoplasm were the endoplasmic reticulum (ER), ribosomes, polyribosmes, Golgi complex, mitochondria, lysosomes, peroxisomes and vacuoles.

Two types of ER (Plate 10) were observed and these were the smooth endoplasmic reticulum (SER), and the rough endoplasmic reticulum (RER). The SER was well developed and widely spread in the cytoplasm of some glandular cells of an HCG acinus, and were either vesiculated or tubular or both. The single membrane bound cisternae were electronlucent and interconnected with those of RER and the perinuclear space. There was swarming profile of RER in some cells of the HCG acinus especially in the adult dog. Ribososmes were found in small clusters or aggregates while some ribosomes appeared bound to the membrane of RER.

The Golgi complex was observed in a non-specific position of the cytoplasm. It had several membranous, flattened saccules or cisternae stacked in a parallel array. The lumina of the cisternae were narrow in their central portion but appeared dilated or expanded toward the ends. The cisternae were often curved so that the organelle as a whole had concave and convex surfaces. Associated with the Golgi apparatus were the low electron dense vesicles (Plate 8).

Mitochondria were large, and numerous with poorly developed cristae in the cells of the HCG. They were observed as double membrane bounded organelles with electrondense matrix. The mitochondria were pleomorphic in shape ranging from ovoid to spherical (Plate 9).

The lysosomes were of both primary and secondary varieties. The primary lysosomes were ovoid in shape with a single limiting membrane. Their matrix was highly electrondense. Secondary lysosomes were observed as myelinated bodies or autophagic bodies or lipofuscin granules. The myelinated bodies were membrane-bound concentric layers of a highly electronlucent membranous structure. The peroxisomes or microbodies were found in the cells of HCG and they were pleomorphic in shape ranging from ovoid to semilunar. They had a single limiting membrane with lower eletrondense fine granular matrices. Lipid vacuoles were observed in the cytoplasm of HCG cells of the puppies but not in the adult gland (Plate 10).
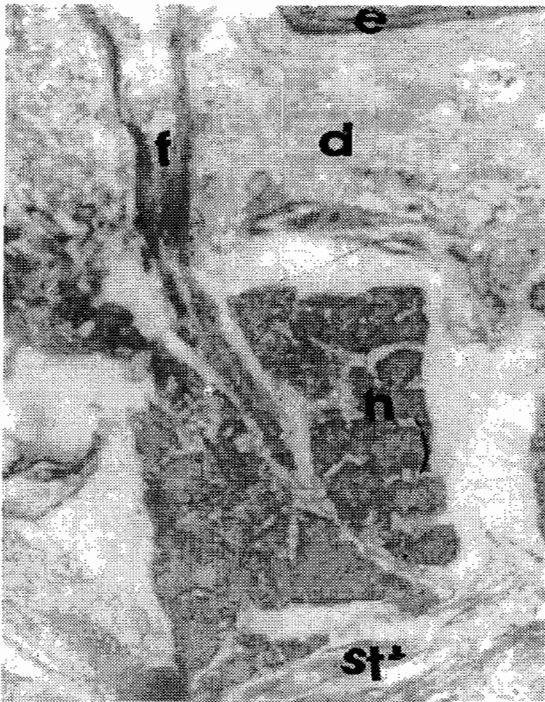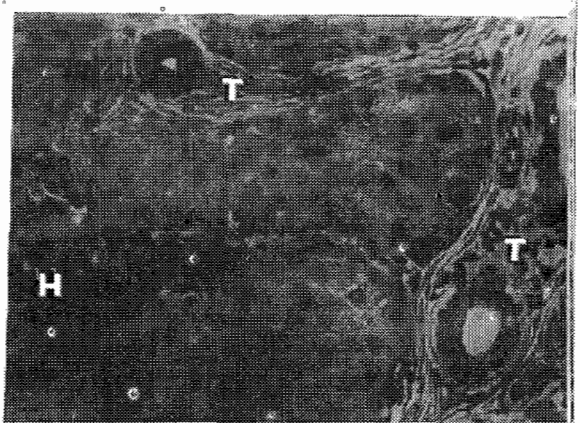
PLATE 1: Light micrograph showing two HCGs separated by a hair follicle in the centre. D – Dermis, e –Epidermis, f -Hair follicle, h – Hepatoidcircumanal gland, st -Striated muscle. LM – X 63


PLATE 2: Light micrograph of A Mixed Gland – Bipartite/Transitional. HCG, F - Hair follicle. H - Deep Hepatoid circumanal gland, Sg – Superficial sebaceous gland. P - Pilosebaceous canal. LM - X 63

PLATE 3: Light micrograph showing HCG lobules with its adjacent connective tissue. H - Cells of hepatoid lobule, T - Ducts of sweat glands, CT -Connective tissue. LM №400
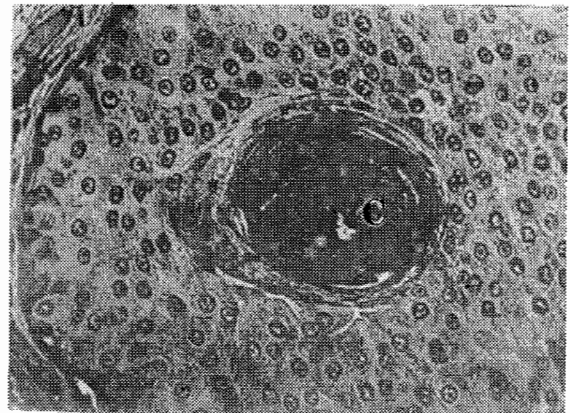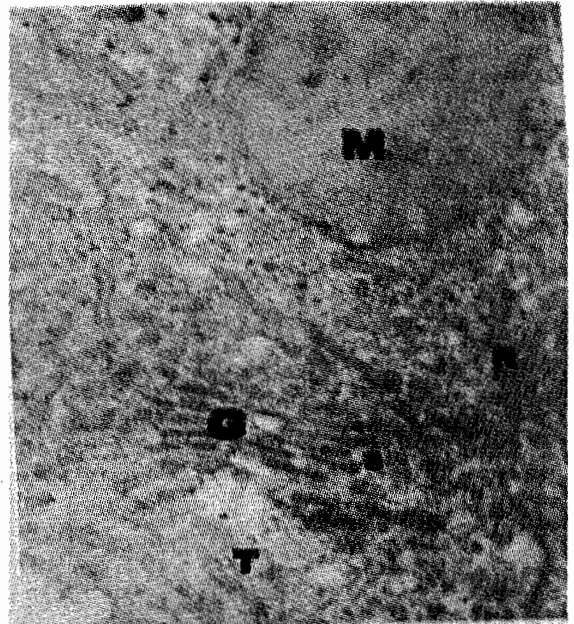

PLATE 4: Light micrograph of a HCG of an adult dog. C -Intralobular cyst in the centre of an HCG lobule. LM- X 250
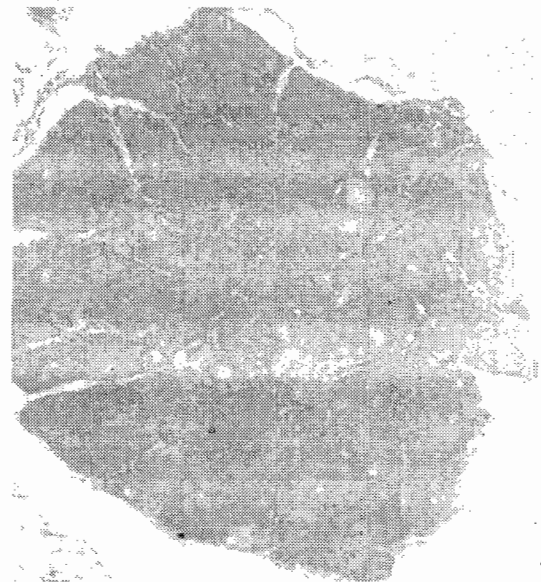

**PLATE 5:** Light micrograph of a complete HCG gland LM - X 63

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PLATE 6: Electron micrograph of a HCG cell.
M – Mitochondria, N- Nucleus, P- Polyribosomes,
S - Smooth endoplasmic reticulum, T- Tonofilaments.
EM- X 19,000

PLATE 8: Electron micrograph showing part of the
cytoplasm of a HCG. G – Golgi apparatus, M –
Mitochondrion, R - Rough endoplasmic reticulum, S - Golgi
vesicle, T - Smooth endoplasmic reticulum. EM - X 42,930
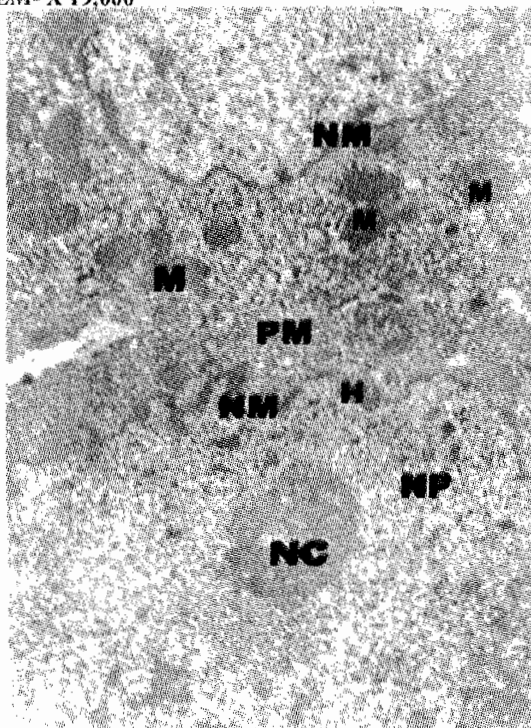
PLATE 7: Electron Micrograph showing two
HCG cells. H – Heterochromatin, NC –Nucleolus,
NM – Nuclear membrane, NP – Nucleoplasm, M –
Mitochondria, PM - Plasma membrane. EM - X
13,080
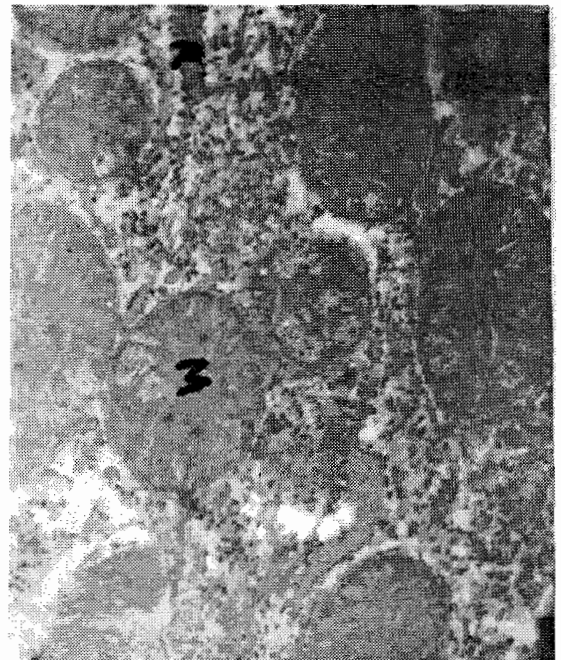
PLATE 9: Electron micrograph showing part of the
glandular cytoplasm of a HCG cell. M -
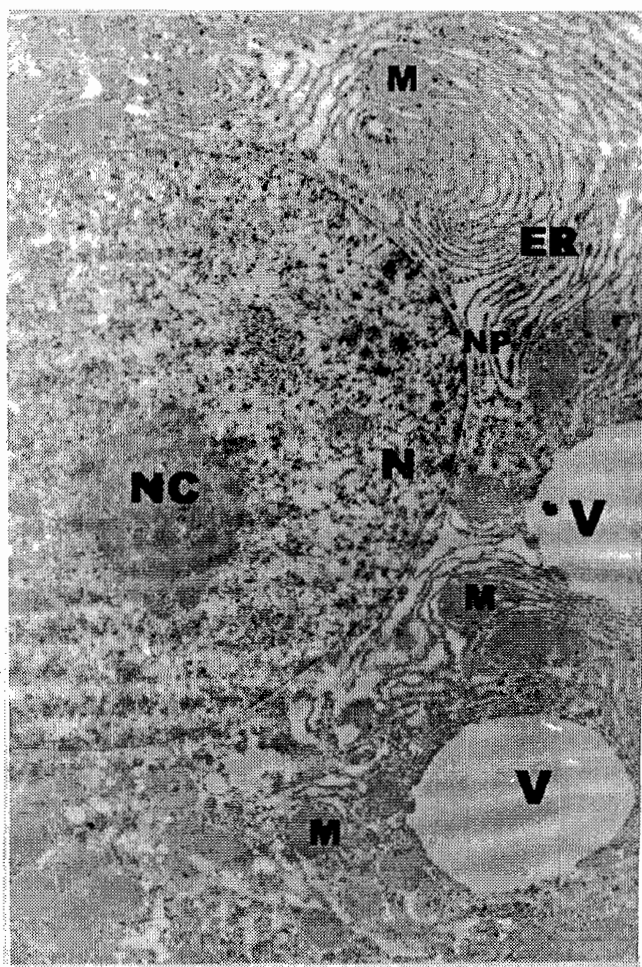Mitochondrion, R - Rough endoplasmic reticulum.
EM- X 42,980

PLATE 10: Electron micrograph of an HCG Cell. ER – Endoplasmic reticulum, M – Mitochondria, N – Nucleus, NC Nucleolus, NP – Nuclear pore, V – Vacuoles. EM - X 7,020

DISCUSSION

The report of earlier workers (Akayevski, 1975; Shabadash and Zelikina, 1993) on exotic breeds of dogs on the presence of hepatoid circumanal glands in the canine perianal skin of both puppies and adult dogs is consistent with the observation of this study and contrary to the report considering the HCGs to be circumanal bodies instead of glands (Atoji et al., 1998a).

The observation that the HCGs are located at the dermo-hypodermal junction (Isitor, 1983; Shabadash and Zelikina, 1993), agrees with the observation of the current study. Nielsen's report of 1953 that HCGs are superficially located in the puppies and deeper in the corium in the adult dog coincides with the observation of this study. The location of these glands at the deep layer of the dermis in the adult agrees with Isitor's report of 1983, which referred to them as dermal skin glands, although the age group was not included.

The presence of striated muscle fibres at the zone of HCGs in this study coincides with the report of Baker (1967) and Isitor (1978). Although, these authors did not discuss the functional importance of the striated muscle fibres in this region, the current study is of the opinion that the fibres are part of external anal sphincter muscle, which is responsible in maintaining faecal continence. External and internal anal sphincter muscles are primarily responsible for maintaining faecal continence at rest and when continence is threatened (Bharucha, 2006).

The classification of HCGs has been controversial. Shabadash and Zelikina (2002a, b, and c) reported that the HCGs are exocrine glands with extracellular secretory canaliculi forming excretory ducts while Nielsen and Aftosmis (1964), Isitor (1983) and Atoji et al., (1998b) described the HCGs as solid, lobulated ductless glands. The observation of the current study coincides with the latter report. The tubular structures observed in the connective tissue adjacent to the HCGs have been reported by Isitor (1983), Schiffman-Wyttenbach, et al. (1983) and Konig et al. (1985) to be ducts of sweat glands. These reports tally with the observation of the current study. HCG cells were found to be polygonal or polyhedral as was previously described (Calhoun and Stinson 1981, Atoji et al. 1998a). The observation that there were light and dark glandular cells and some of these cells had more than one nucleolus agrees with Isitor's findings of 1983.

Shabadash and Zelikina (1993) reported that the HCGs in puppies were represented by two histological varieties. The observation of the current study agrees with this report since the first variety had superficial vacuolated sebaceous-like cells in some lobules with a deeper portion consisting of compact, solid, non vacuolated cells. Parks (1950) referred to this type of structure as "bipartite structure", and Isitor, (1979) and Konig et al. (1985) called it transitional HCG. The

second variety had few vacuolated sebaceouslike cells in the centre of a lobe that was surrounded by compact, non vacuolated glandular cells of HCG lobules. This may correspond to what Isitor, (1978) Isitor and Weiman (1979) referred to as the duct of the gland laden with fat.

The reports that HCGs of adult dogs are characterized by only one variety forming cysts tallies with the observation of the current study (Isitor, 1983; Shabadash and Zelikina, 1991, 1995). Smooth muscle fibres were not observed in the current study. This observation agrees with the report of Baker (1967) whereas Isitor (1978) reported to the contrary the presence of smooth muscle fibres. Our finding agrees with earlier report that the lobules were surrounded by large blood vessels and capillaries.

Controversy existed as to the function of HCGs. According to Parks, (1950) HCGs showed no functional activity. Workers like Shabadash and Zelikina (2002a, b) are of the opinion that HCGs are odoriferous exocrine glands producing and secreting protein rich substances in adults and hydrophobic lipidrich substances in puppies. Contrary to Shabadash and Zelikina (2000c), who reported the presence of ducts and extracellular canaliculi, the current study failed to observe any evidence of ducts associated with HCGs. Others have reported HCGs to be ductless glands involved in steroid metabolism (Atoji *et al.* 1998a; Petterino *et al.* 2006; Pisani *et al.* 2006).

In the current study, the ultrastructural feature of some HCG cells with their swarming profile of RER suggests that these glands may be involved in protein metabolism and the well developed SER in other cells of HCG is suggestive of their involvement in lipid metabolism. The importance of HCGs has been well documented in neoplasia in Europe and America. Transmissible venereal tumour is prevalent in Nigerian dogs. More work needs to be done to elucidate the normal functional morphology of the HCGs and their role in transmissible venereal tumour in Nigerian adult male dogs.

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