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Ultrastructural changes in the testes of prepubertal to aged African Greater Cane rat (*Thryonomys swinderianus*, Temminck 1827)

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Abstract

This study investigated ultrastructural changes in the testes of prepubertal to aged African Greater Cane rats (*Thryonomys swinderianus*). Twenty, pathogen-free AGCRs of known ages were used for this study. The rats were randomly assigned into 4 groups (n = 5) as follows: Groups 1 (prepubertal), 2 (pubertal), 3 (adult) and 4 (aged). Testicular tissues were processed routinely and viewed under a Transmission Electron Microscope. Sertoli cell nuclei were uniquely roundish in prepubertal compared to their triangular shape in others. Also, spermatogonia and spermatocytes in prepubertal had more mitochondria and euchromatic nuclei compared to others. The Leydig cell cytoplasm in pubertal rats contained numerous mitochondria, and lipid droplets as well as the smooth endoplasmic reticulum when compared to others. This study has demonstrated specific ultrastructural changes in the testes of the African Greater Cane rat with age advancement that could perhaps be associated with the reproductive status; quiescence or activeness of different age groups of the rats investigated.

Keywords: Testes, ultrastructural changes, *Thryonomys swinderianus*

Running title: Age-related changes in the testicular ultrastructure of cane rat

Introduction

The mammalian testis is structurally constituted into two major compartments: the intertubular or interstitial compartments between seminiferous tubules in a lobule housing blood and lymphatic vessels, nerves, connective tissue cells, besides macrophages, mastocytes, and Leydig cells and tubular compartment which houses the seminiferous tubules (the seat of spermatogenesis) (Costal *et al*., 2006). The individual seminiferous tubule within the testicular parenchyma is enveloped or bounded by peritubular tissue in all mammalian species (Maekawa *et al*., 1996). The organization of peritubular tissues appear to be specie-specific and the fact that it is widely distributed among different mammalian species implies that boundary tissue is an essential testicular constituent (Maekawa *et al*., 1996).

In between the seminiferous peritubular tissue and the tubular lumen, several cells of various sizes and shapes representing stages in the formation of spermatozoa are found and are collectively referred to as germ cells (Singh, 2011). The germ cells are flanked by the sustentacular cells, the Sertoli cells (Young *et al*., 2006). In mammals at puberty onwards, the germ cells consist of sets of spermatogonia, spermatocytes, spermatids (round and elongating) and spermatozoa (Beguelini *et al*., 2009). Seminiferous epithelium in newborn mammals consists of two different cell types; gonocytes and Sertoli cells. The gonocytes are large round cells (approximately 20 - 24 µm in diameter) evident at the centre of the seminiferous cords. They bear spherical nuclei containing homogenous chromatin, and centrally placed filamentous nucleoli as well as low spherical cytoplasmic mitochondria (Bellve *et al*., 1977).

The African Greater Cane rat (*Thryonomys swinderianus* Temminck, 1827) (AGCR) otherwise referred to as grasscutter or marsh cane rat is a grass-eating rodent found in the rainforest of sub-Sahara Africa (Monadjem *et al*., 2015). It remains the second largest rodent of African origin after the Cape porcupine (Skinner and Smithers, 1990). The male cane rat attains sexual maturity at about 8 months of age and can live up to 4 years in captivity (Soro *et al*., 2014). It contributes to the domestic and foreign earnings of most countries in the South of the Sahara where its meat is sold and preferentially demanded over other wild rodents (Ntiamoa-Baidu, 1998; Asibey and Addo, 2000). Except for the report of Adebayo et al. (2019) on acrosomal formation within the testes of this animal, there is no detailed documentation on the testicular ultrastructural changes in the different age groups of African Greater Cane rats. Hence, this study seeks to investigate age-related

changes in the testicular ultrastructure of African Greater Cane rats.

Materials and Methods

Animals

Twelve clinically healthy male African Greater Cane rats of known ages procured from a cane rat farm, Ibereko, Badagry, Lagos state, Nigeria were used for this study. The cane rats were acclimatized for 7days in wired cages within the experimental animal facility of the Faculty of Veterinary Medicine. They were fed on dry corn daily and water was provided *ad libitum*. The use of AGCR for this experimental protocol was approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC) and was assigned an ethical clearance certificate (UI-ACUREC/18/0120).

Experimental Design

The age grouping from our previous study Omirinde *et al.* (2019) was adopted. Briefly, procured cane rats were randomly divided into four groups (n = 3) as shown below: Group 1: Prepubertal (Pre), ≤4 months, Group 2: Pubertal (Pub), >4≤12, Group 3. (Adult); >12≤30 months and Group 4: aged (AG); >30 months. On day 8th of acclimatization, combined sedative agents; xylazine and ketamine (20:80 mg/kg body weight correspondingly) were injected intramuscularly to the rats for sedation. Thereafter, intracardial perfusion was carried out with Karnovsky glutaraldehyde fluid [A mixture of paraformaldehyde (2g powder in 25 ml of distilled water), 1M Sodium hydroxide (2-4 drops), 25% glutaraldehyde (10ml) and 0.2M of cacodylate buffer (20ml)].

Processing of cane rat testicular tissues for electron microscopy

Glutaraldehyde (Karnovsky)-fixed testicular tissues were routinely processed for TEM as earlier reported in our previous work on the epididymis Omirinde *et al*. (2020). Semi-thin sections of the testes were stained with toluidine blue and viewed under the light microscope (Olympus BX63 with a DP72 camera). After this, ultra-thin sections were stained in uranyl acetate and lead citrate. The copper grids were viewed under a transmission electron microscope (Philips CM 10 TEM, USA) operating at 80 kv. Micrographs of the testicular components (boundary tissues, Sertoli cell, spermatogonia, spermatocytes and Leydig cells) were captured using a Gatan 785 Erlangshen digital camera (Gatan Inc., Warrendale PA).

Results

Changes in the testicular ultrastructure with age advancement

Age-related changes were sequentially observed from the wall of the seminiferous tubule to the core of the parenchyma as follows:

Peritubular tissue (Boundary tissue)

Testicular boundary tissue component was observed to be similar across the different age groups of AGCR investigated (Fig. 1). It is made up of the basal lamina, an additional 4 basal lamina-like structures separated by a single layer of myoid cells, collagen fibres and microfilament substances.

Sertoli cell

The Sertoli cells of the different AGCR groups were seen close to the basement membrane and extended towards the tubular lumen (Fig. 2). It visibly forms junctional complexes around germ cells (Figure 2, Sertoli cell perinuclear aspect). The Sertoli cell contained a roundish nucleus in the pre-pubertal rat (Fig. 2A) while in pubertal to aged AGCR, it is more triangular in shape with a conspicuous nuclear cleft evident in the adult Sertoli cell (Fig. 2C).

Spermatogonia

Three distinct spermatogonia types; Type A, Intermediate and Type B were identified close to the basal lamina from pre-pubertal rats onwards (Fig. 3). The nuclear chromatin nature, the presence as well as the location of nucleoli within spermatogonia were used to morphologically identify the spermatogonia types. Type A spermatogonia in all AGCR groups (Fig. 3) was characterized by oval nuclei that are devoid of nucleoli, the presence of mitochondria and interdigitations with basal lamina in all the groups. However, the nucleus in pre-pubertal was more euchromatic than others (Fig. 3A). Type B spermatogonia contained centrally positioned nucleoli with some degree of nuclear chromatin condensation (Fig. 3). Numerous mitochondria were seen in the cytoplasm of type B spermatogonia in pre-pubertal rat as well as increased nuclear euchromasia when compared to others (Fig. 3A). Intermediate spermatogonia were identified by the presence of some degree of chromatin condensation and with presence of nucleoli that were almost approaching the centre of the nuclei (Fig. 3).

Spermatocyte

Ultrastructurally, five types of spermatocyte (pre-leptotene, leptotene, zygotene, pachytene and diplotene) were recognized within the seminiferous epithelium of the different age groups of AGCR (Fig. 4). Preleptotene in all AGCR was characterized by spherical nucleus with granular chromatin and reduced cytoplasmic organelles except for prepubertal rat with intense euchromasia and conspicuous presence of many mitochondria (Fig. 4). Leptotene spermatocyte was observed to have

spherical nucleus with well-defined nuclear membrane and less chromatin condensation in all groups except for pre-pubertal rat with a somewhat ellipsoidal nuclear shape, intense nuclear euchromasia and numerous tubular mitochondria (Fig. 4). Zygotene in the different age groups of AGCR was observed to have reduced cytoplasm, less prominent synaptonemal complexes, more nuclear heterochromasia from pubertal to aged relative to the pre-pubertal rat (Fig. 4). There was also increased mitochondrial presence in the cytoplasm of zygotene in the pre-pubertal rat compared to others (Fig. 4A). The pachytene spermatocyte of all AGCR had prominent nucleolus, synaptonemal complex and extensive cytoplasm (Fig. 4). The pre-pubertal rats had numerous mitochondria in their cytoplasm when compared to others (Fig. 4A). Diplotene spermatocyte in all AGCR was characterized by deep nuclear chromatins which were aggregated to one side of the nucleus and the presence of nucleoli (Fig. 4). The numerous mitochondria and higher degree of nuclear euchromasia consistently seen in the earlier listed spermatocytes of pre-pubertal were also observed in this spermatocyte (Fig. 4A).

Leydig cell

Except for the scanty nature of the Leydig cells in the interstitium of pre-pubertal rats, the interstitial spaces in pubertal to aged AGCR were filled with Leydig cells (Fig. 5). It was observed that the Leydig cell was generally ovoid in outline and had a roundish nucleus which in pre-pubertal contains greater amount of heterochromatin relative to other groups (Fig. 5A). Also, Leydig cell cytoplasm in pubertal rat contained numerous mitochondria, lipid droplets as well as smooth endoplasmic reticulum when compared to others (Fig. 5B). Numerous stacks of concentric rough endoplasmic reticulum and smooth endoplasmic reticulum were seen in the cytoplasm of Leydig cell in adult and aged AGCR (Fig. 5C and D).

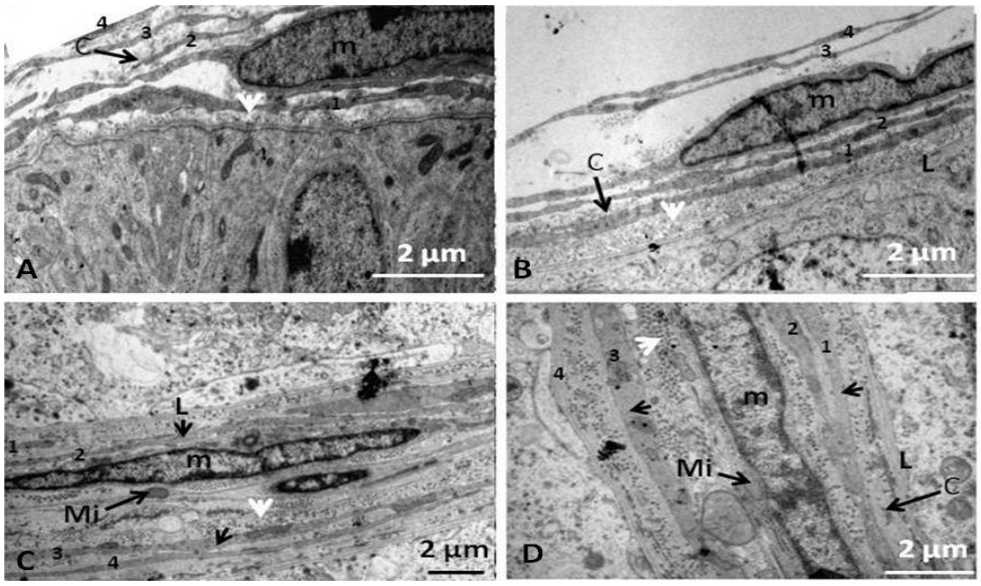

Fig. 1. Transmission electron micrographs of the testicular boundary tissue of the African Greater Cane rat. A. Prepubertal B. Juvenile C. Adult D. Aged. Note the basal lamina (L) as well as the several layers (1 - 4) of basal lamina-like structures (arrowheads) separated by myoid cells (m), collagen fibrils – C and Microfilament (White arrow-head). Mi – Mitochondria.

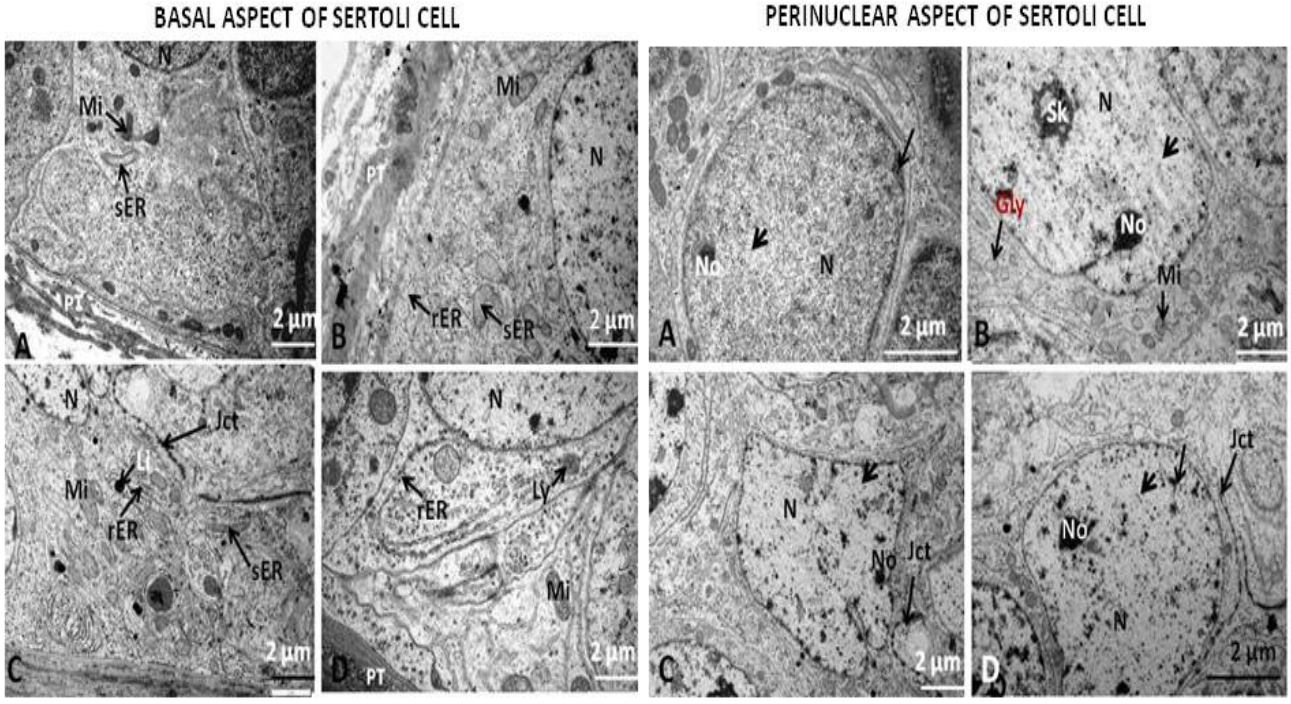

Fig. 2. Transmission electron micrographs of the basal and perinuclear aspects of Sertoli cell of African Greater Cane rat. A. Prepubertal B. Juvenile C. Adult D. Aged. Note in the perinuclear aspect of Sertoli cell the intense nuclear heterochromatin (arrow) in the Sertoli cell nucleus (N) of A. rER - Rough endoplasmic reticulum, sER - Smooth endoplasmic reticulum, N - Sertoli cell nucleus, No - Nucleolus, Sk - Satellite karyosomes, NC - Nuclear cleft, arrowhead - euchromatin Mi- Mitochondrial, Ly - Lysosome, Li - Lipid droplet, Jct - Sertoli cell junction, m - Myoid cell, PT - Peritubular tissue.

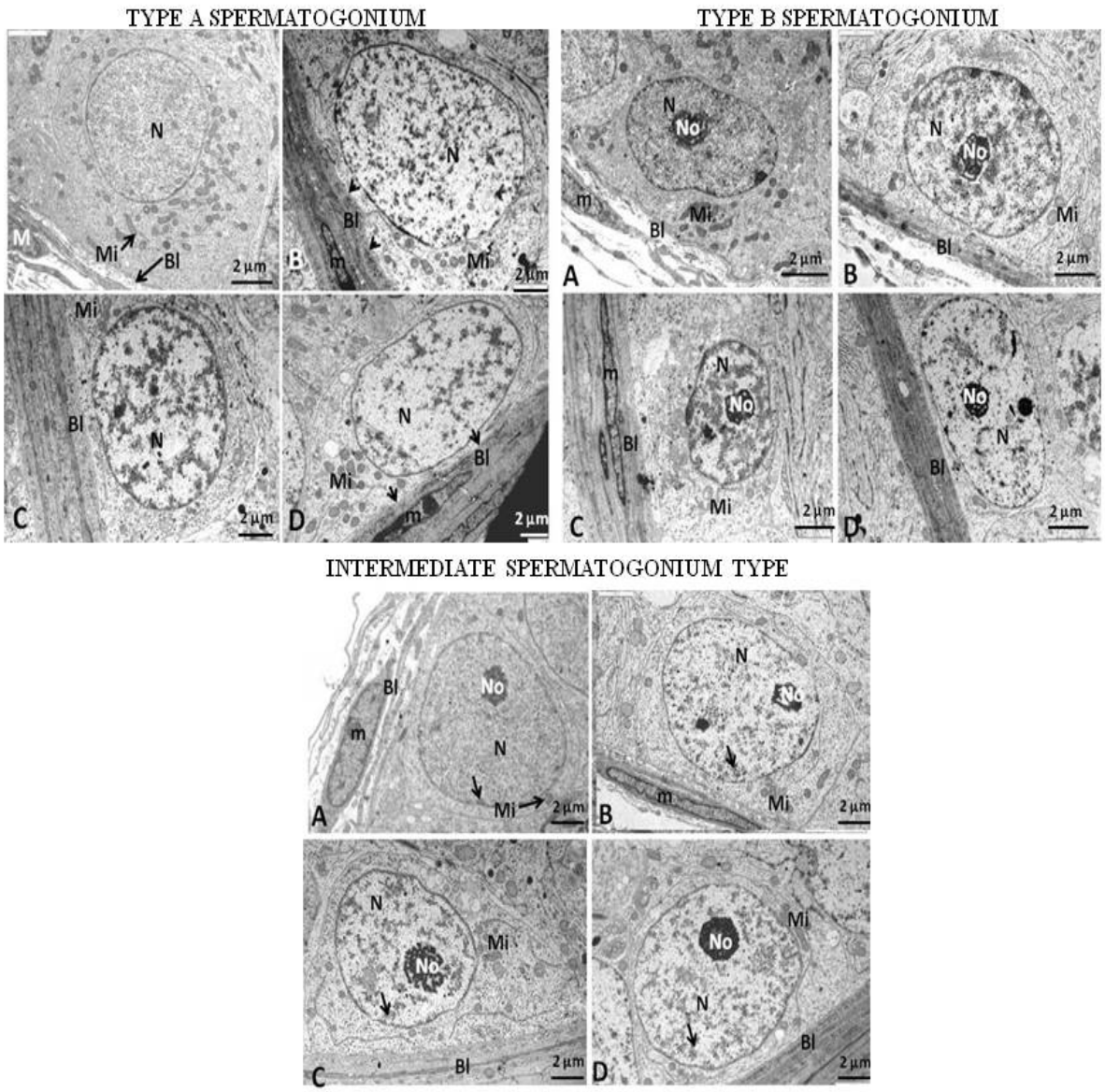

Fig. 3. Transmission electron micrographs of the spermatogonia in the testis of the African Greater Cane rat. A. Prepubertal B. Juvenile C. Adult D. Aged. **Type A spermatogonium:** bears oval nuclei (N) devoid of nucleoli, the presence of mitochondria (Mi) and interdigitations (arrowhead) with basal lamina (Bl) in all the groups as well as more euchromatic nucleus in A and numerous mitochondria in the cytoplasm. **Type B spermatogonium:** has centrally positioned nucleoli (No), marked euchromatic nucleus in A as well as conspicuous nuclear heterochromasia in B - D (arrow). **Intermediate spermatogonium:** has condensed nuclear chromatin (arrow) and nucleoli (No) approaching the centre of the nuclei (N). Bl - Basal lamina, M - Myoid cell.

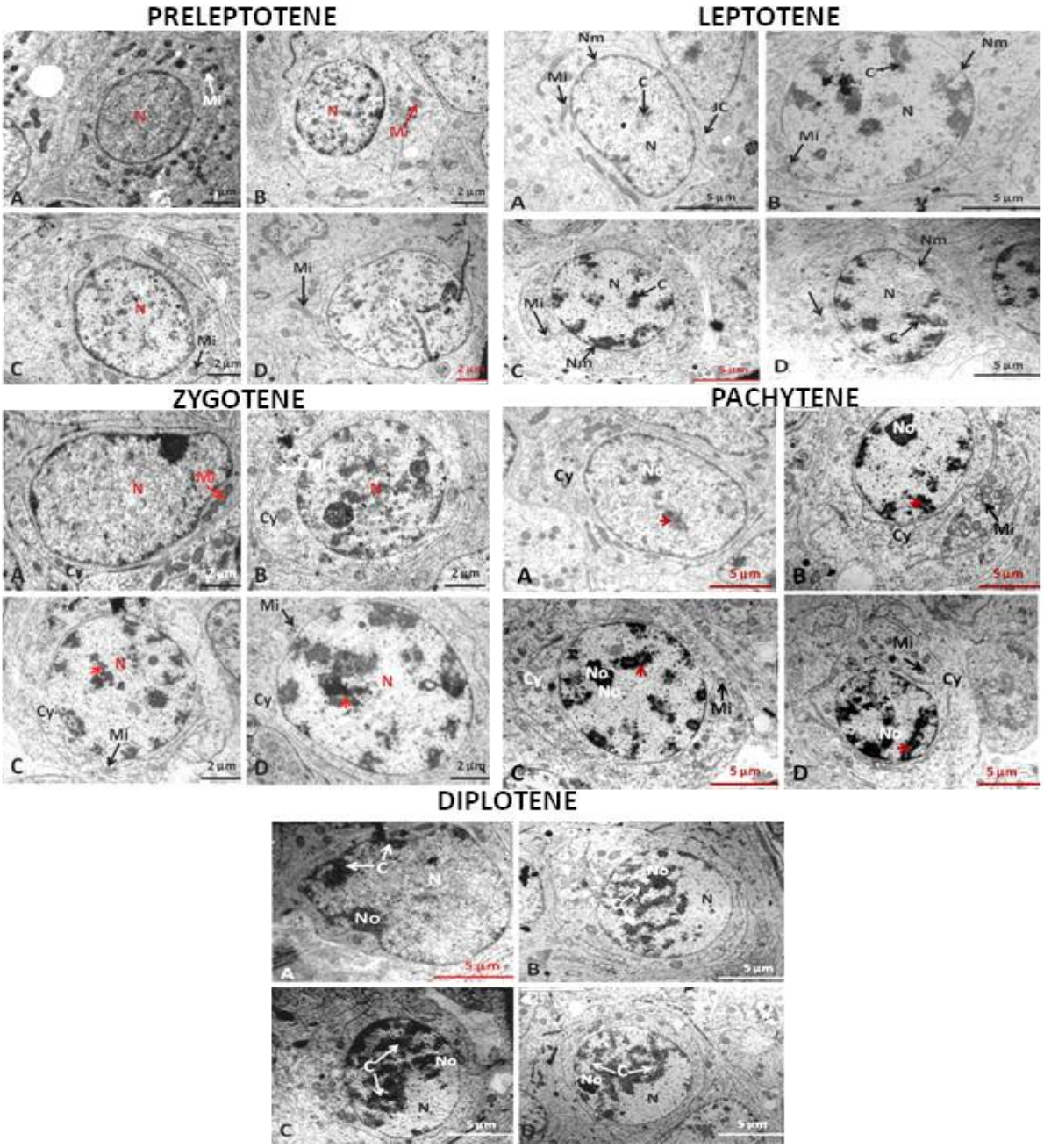

Fig. 4. Transmission electron micrographs of the spermatocytes of the African Greater Cane rat. A. Prepubertal B. Pubertal C. Adult D. Aged. **Preleptotene:** note the presence of fine granular chromatin in the nucleus (N) and clear cytoplasm (Cy) across all age groups. **Leptotene:** has a spherical nucleus (N) with a well-defined nuclear membrane (Nm) and less chromatin (C) condensation in all groups except for the somewhat ellipsoidal nuclear (N) shape of pre-pubertal. Also, note the numerous tubular mitochondria in A. **Zygotene:** note the reduced cytoplasm (Cy) across groups as well as less prominent synaptonemal complexes (arrowhead). **Pachytene:** has prominent nucleolus (No), synaptonemal complex (arrowhead) and extensive cytoplasm (Cy). **Diplotene:** note the aggregation of the deep chromatin (C) towards one side of the nucleus and the prominence of nucleoli (No). Also, observe the irregular shape of the nucleus (N) in A as well as high nuclear heterochromatin. JC - Junctional complex.

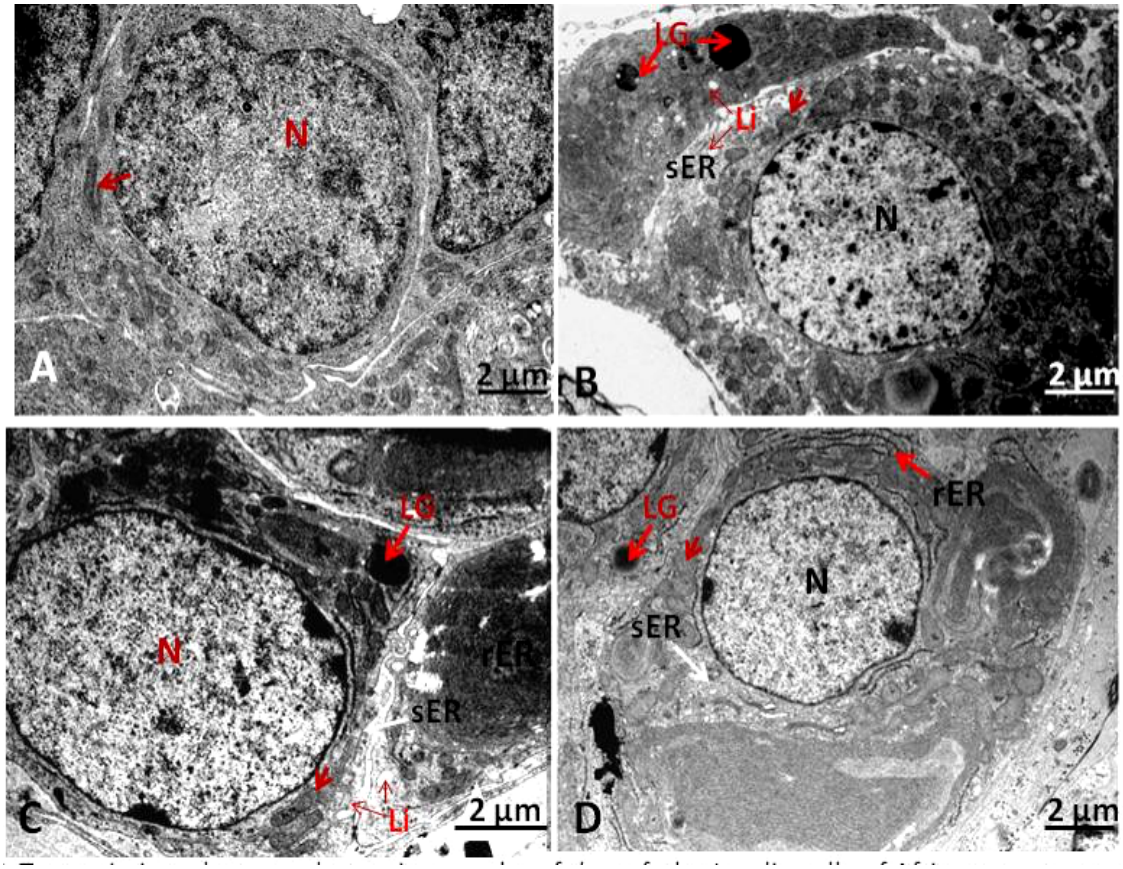

Fig. 5. Transmission electron micrographs of the Leydig cells of the AGCR. A. Prepubertal: B. Pubertal: C. Adult: D. Aged. Note the great number of mitochondria (arrowhead), lipid droplets (Li), and smooth endoplasmic reticulum (sER) in the Leydig cell (LC) cytoplasm of pubertal AGCR relative to others. Also, observe the reduced organelles in A compared to others and the numerous stacks of concentric rough endoplasmic reticulum (rER) in the cytoplasm of the adult and aged AGCR LC. LG - Lipofuschin granule.

Discussion

The presence of similar testicular boundary tissue components in all the age categories of cane rats is suggestive of the morphophysiological roles of mechanical support, spermatozoa discharge and as a barrier for regulating material movements across the parenchyma (Marettova *et al*., 2010). The ultrastructural components of testicular boundary tissue observed across all age categories of the cane rats agree with the boundary tissue composition in rodents (Maekawa *et al*., 1996, Rezigalla *et al*., 2012). However, it is incongruent with reports of numerous myoid cell layers present in the boundary tissue described in large animals (Virtanen *et al*., 1986, Maekawa *et al*., 1996) and in avian spps (Aire, 1997; Aire and Ozegbe, 2007).

The roundish shape of the Sertoli cell nucleus observed in the pre-pubertal testes of the cane

rats unlike the typical triangular shape in the other groups is supported by the fact that Sertoli cell can assume several different shapes depending on the stage of the seminiferous cycle and the age of development (Russell *et al*., 1990, Hess and Franca, 2005). Regarding Sertoli cell nuclear location, the presence of the nucleus close to the basal lamina even with ageing in different groups of the AGCR is comparable to the Sertoli cell nuclear position in the Agoutis rodent (Arroyo *et al*., 2015) but is at variance with a distant location in Spix's yellow-toothed cavy rodent reported by Santos *et al.* (2014). Besides the nuclear profile, the Sertoli cell cytoplasm especially in the adult AGCR was remarkably observed to contain abundant SER which has been previously postulated by Hess and Franca (2005) to have a functional correlation with lipid or steroid metabolism.

The identification of three distinct spermatogonia types (Type A, Intermediate and B) in the proliferative phase of spermatogenesis close to the basal lamina in the prepubertal group onwards could be linked to the essential role of spermatogonia in reproduction. Spermatogonia are recognized stem cells that are important in the preservation of the spermatogenetic process through their proliferative potential that culminates in the production of numerous spermatozoa (Phillips et al., 2010). Hence, the occurrence of spermatogonia across all age groups underscores the above-highlighted function. This finding is in agreement with reports from similar age-related studies (Assis-Neto *et al*., 2003b, Arroyo *et al*., 2015). The consistent observation of euchromatic nuclear type in the spermatogonia of pre-pubertal rats could be indicative of active transcription activity (Feher, 2012). In addition, the presence of numerous mitochondria in the cytoplasm of different spermatogonia types of the pre-pubertal rat could be suggestive of high metabolic activities.

The identification of five spermatocyte types; Pre-leptotene, leptotene, zygotene, pachytene, and diplotene characterized by progressive increase in nuclear size, synaptonemal formation and chromatin condensation in the meiotic phase of spermatogenesis in different cane rat groups is consistent with spermatocyte types described in mammals (Hunter, 2003; Page and Hawley, 2004; Beguelini *et al*., 2011) and in age-related study by Arroyo *et al*. (2015). The irregular nuclear and cytoplasmic shapes that typify the prepubertal spermatocytes could be associated with rapid meiotic cellular activity in this age group. The observed ultrastructural alteration in the prepubertal spermatocyte morphology concurs with the report of Bellve *et al.* (1977) on prepubertal mice.

The observed lower Leydig cell population and fewer mitochondria in the pre-pubertal rat

compared to others are suggestive of less reproductive activity in this age group and with advancement in age remarkable features of active reproduction would be evident. These findings are similar to the reports of Tripepi *et al*. (2000) in pigs and Lasheen *et al*. (2015) in rats.

The remarkable increase in the number of lipid droplets and SER observed in the Leydig cell cytoplasm of adult AGCR could be associated and consistent with the expected high steroidogenic activity. These findings corroborate the morphological features reported for ground squirrels at full spermatogenesis (Pudney *et al.*, 1985).

Conclusion

This study has brought to the fore specific ultrastructural changes in the testes of the African Greater Cane rat with age advancement that could perhaps be associated with the reproductive status; quiescence or activeness of different age groups of the rats investigated. We hope that the next research unravels the possible age-related ultrastructural changes in the spermatids to mature spermatozoa of this animal.

Conflict of interest

We declared that there is no conflict of interest.

Acknowledgement

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