HISTOLOGICAL STUDIES OF SOME ORGANS OF SQUIRRELS (*Xerus erythropus*) IN TROPICAL ECOLOGICAL ZONE

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ABSTRACT

The functional morphology of some organs of squirrels was investigated through histological observations. A total number of thirty two (32) ground squirrels (Xerus erythropus); 15 males with mean weight 220.0 ± 2.0 g and length 40.0 ± 0.2 cm, and 15 females with mean weight 229.8 ± 2.0 g and length 39.0 ± 2.0 cm were used. The ground squirrels were trapped using rodent traps, anesthetized identified to species level and dissected to expose the viscera. The organs (liver brain, heart, gonads (testes and ovaries) were dissected out, clean off ceolomic fluid, fixed in normal buffered saline prior to the histological studies of the tissues. The organs of squirrel from different zones were examined at the light microscopy. The results compared clearly showed normal tissues without degenerations, the tissue histology showed almost the same pattern and arrangement of the cells in the tissues. The liver develops embryologically outgrowth of the gut. The endocardium, with all its endothelial lining and supporting tissues, accommodates movement of the myocardium. The cerebellar cortex forms a series of deeply convoluted folds or folia. The spermatozoon is absorbed into the lumen of the tubule, where they are drawn into the epididymal walls of the testis while the primary oocyte is surrounded by a single layer of flattened follicular cells.

Keywords: Histology, Liver, Heart, Brain, Testes, Ovary, Squirrel

INTRODUCTION

Squirrels belong to a large family of small or medium-sized rodents called *Sciuridae* (Steppan *et al.,* 2004) which includes; tree squirrels, ground squirrels (Friggens, 2002), chipmunks (Baack, and Paul, 2003), marmots (including woodchucks), flying squirrels, and prairie dogs (Foltz and Hoogland 1981). Ground-dwelling species are generally social animals, often living in well-developed colonies, but the tree-dwelling species are more solitary (Milton, 1984). The ground species (*Xerus erythropus)* are typically diurnal, while flying squirrels tend to be nocturnal - except lactating flying squirrels and their offspring, which have a period of diurnality during the summer (Thorington and Hoffman, 2005). They are cosmopolitan in their distribution, except in the Polar Regions. In the wild, most species of squirrels are threatened or endangered. Human activities are responsible for direct (i.e. hunting squirrel for food and trapping of same for research or display), or indirect (i.e. habitat destruction) decimation of wild populations. Although about 9,000 squirrels are still being imported into the United States of America annually for research, there is increasing emphasis on obtaining "bred for purpose" animals for research. Squirrels feed primarily on seeds and plant. Difference in diet is thought to be a key factor responsible for the rapid increase of squirrel distribution, and may significantly or partially explain their success. Specifically, the meat of squirrel is usually considered a favoured meat in certain regions of the United States and the United Kingdom, where it is listed as wild game. The meat can also be exchanged for rabbit or chicken in some recipes as the meat is low in fat content, unlike most game meat (Bradley, 1968; Davidson, 1999; Muser, 2007; Musser *et al*., 2010).

Thus, a thorough knowledge of normal histology is essential for the understanding of the altered structure seen in the various conditions of disease (Copenhaver, 1964; Banks, 1993). Diseases can also be a significant problem; with squirrel pox virus (sometimes colloquially referred to as parapox or simply pox), caused by a protozoan parasite *Toxoplasma gondii* being common and mange ringworm being quite rare. Mortality varies between species and populations and is strongly correlated with the mast crop, with higher survival during good mast years. Mortality sources for squirrels include predators (domestic dogs and cats), starvation and road vehicles, with the latter being the most recent common in the Isle of Wight where out of 158 animals found dead between September 2008 and October 2009, 123 (78%) had been hit by cars (Wikipedia, 2010). Liver disease is not very common in squirrel. Multinucleated hepatocytes are occasionally seen as an incidental finding in squirrel as well as in chimpanzees and gorillas. Hepatic hemosiderosis is a common "incidental" finding in marmosets, tamarins, owl monkeys and other species of New World primates and in lemurs and gorillas (Lowenstine, 2003). Experimentally, diets high in iron caused mortality due to infections is common in marmosets. Hepatocellular iron storage may become severe enough to lead to alterations in hepatocellular function (hemochromatosis) compromising pharmacologic studies (Lowenstine, 2003). In lemurs, hepatocellular iron is thought to play a role in the development of spontaneous hepatocellular carcinomas, but recently a virus has also been identified. The circulatory system is a closed hydraulic system powered by a pump-the highly efficient heart. The micro-architecture of the heart-arteries, veins, and capillaries-is reflected in their functions. The close and strikingly obvious correlation between structure and function in the heart and vessels of the blood vascular system greatly simplifies understanding their histologic organization. Caprette and Senturia (1984) reported that hearts from winter-active ground squirrels developed greater pressures than those from winter-hibernating and summer-active animals. Contractility of the seasonal hibernator's heart is influenced by both season and hibernation itself possibly through shifts in myocardial metabolism. However, seasonal adaptations appear not to be required to confer the special resistance of the seasonal hibernator's heart to the deleterious effects of low temperature. Hoque *et al.* (2011) reported that ground squirrel's brain loses many vital neural connections, but it has evolved a way to recuperate. Understanding that process might help scientists treat Alzheimer's diseases. Evidence from other hypoxic-tolerant species suggests that some of the adaptations are similar but perhaps not sufficient to promote ischemic tolerance. For example, turtle brain is highly resistant to anoxia, but inhibition of glycolysis (as it would occur during ischemia) renders this species highly vulnerable to injury. Hoque *et al*. (2011) reported that during selection of breeding buck, special attention should be given on age, body weight, soundness of the sexual organ especially testis and quality of semen.

The structural alterations are minimized when tissues and cells for microscopic examination are fixed in quality fixatives. The objectives this study was to describe the histological arrangement of tissues of some organs of squirrels (liver, brain, heart and gonads (testes and ovaries)) sampled from four villages (Ede-Oballa, Obukpa, Ibagwa and Eha-alumona) in Nsukka agro-ecological zone.

MATERIALS AND METHODS

The 32 ground squirrels used in this study were wild-caught from four villages in Nsukka in agro-ecological zone. Animals were cared for in accordance with guidelines on animal experimentation of the Committee on Care and Use of Laboratory Animal Resources, under an animal use protocol approved by the University of Nigeria, Nsukka. This concentration on a particular species was a consequence of their commonality and their gregarious nature in these areas. Twenty-two ground squirrel were caught from their dens using specialized catching traps, and eleven with land traps. All specimens were collected between May and July, 2012 from four villages (Ede-Oballa, Obukpa, Ibagwa and Eha-alumona) in Nsukka agro-ecological zone, Enugu, Nigeria. Collected squirrels were identified to species level as *Xerus erythropus* (Milton, 1984). They were then transported to the Histology Laboratory of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, where specimens were anesthetized using chloroform fumes and the viscera dissected to expose the internal organs. The organs were dissected out, cut and fixed in 10% neutral-buffered formalin for 36 hours (Onuoha, 2010) and transferred to 70 - 98% ethanol for dehydration before being processed for routine paraffin embedding, sectioning, staining re-dehydration and mounting (Weiss *et al.*, 2010). Several 4 – 5 µm sections were made from each tissue and stained with the Hematoxylin and Eosin. The tissues were processed using standard histological methods (Onuoha, 2010).

RESULTS

Liver: In squirrels, the liver develops embryologically as a glandular outgrowth of the primitive gut. The liver cells (hepatocytes) are separated by wide vascular channels, sinusoids (S). It has a hepatic portal vein (Figures 1 – 4) from were absorbed food products pass directly from the gut to the liver. The larger branches of the hepatic artery and vein are separated by fibrous tract, the portal tract (T), usually arranged around a terminal hepatic venule. There were no differences in the architecture of the liver tissue of squirrels sampled from the four villages in Nsukka agro-ecological zone (Figures 1 – 4).

Heart: This constitutes the circulatory system of squirrels. It mediates the continuous movement of their body fluids, its principal functions being the transport of oxygen and nutrients to the tissues. It has two functional components, the blood vascular system and the lymph vascular system. It comprises three layers: an inner lining comprising a single layer of extremely flattened cells called endothelium (E), forming the Tunica intima; an intermediate muscular layer, the Tunica media; an outer supporting tissue layer, the Tunica adventitia. Furthermore, the *Tunica intima* forms the endocardium (innermost); the *Tunica media* forms the myocardium, the *Tunica adventitia* forms the epicardium (Epi), enclosed by a fibrous sac, the pericardium (the parietal pericardium) (Figures 5 – 8). The endocardium, with all its endothelial lining and supporting tissues, accommodates movement of the myocardium without damage to the endothelium and may also contain a small amount of adipose tissue for insulation. The mesothelial cells of the epicardium secrete a small amount of serous fluid, which lubricates the movement of the epicardium on the parietal pericardium. There were no differences in the architecture of the heart tissue of squirrels sampled from the four villages in Nsukka agro-ecological zone (Figures 5 – 8).

Brain: In squirrels, the cerebellar cortex forms a series of deeply convoluted folds or folia, supported by a branching central medulla (M) of white matter. It consists of the outer molecular layer (ML) which contains relatively few neurons and large numbers of unmyelinated fibres, the inner granular cell layer (GL) which is extremely cellular Purkinje cells (P) found between the two layers (Figures 9 – 12). It has very large cell bodies, a relatively fine axon extending down

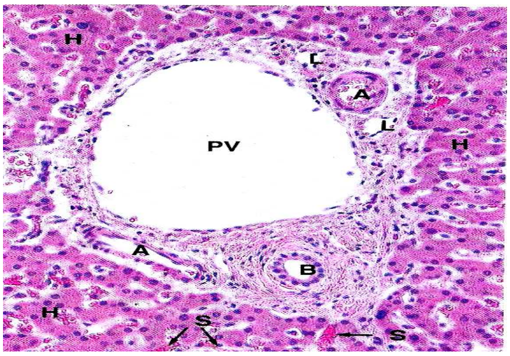

Figure 1: Transverse section of squirrel liver from Ede-Oballa, showing a branch of the hepatic portal vein PV, plates of hepatocytes H, a branch of hepatic artery A, lymphocytes L, hepatic sinusoids S and bile ductulus B. H&E x 100

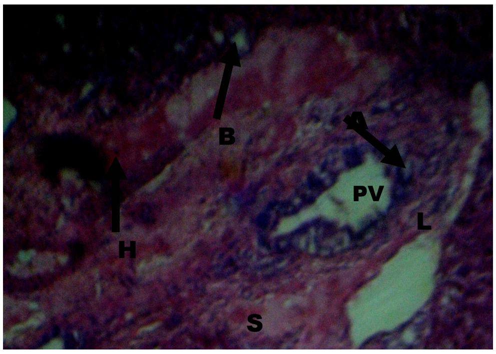

Figure 2: Transverse section of squirrel liver from Eha-Alumona, showing the hepatic portal vein PV, plates of hepatocytes H, lymphocytes L, hepatic sinusoids S and bile ductulus B. H&E x 100

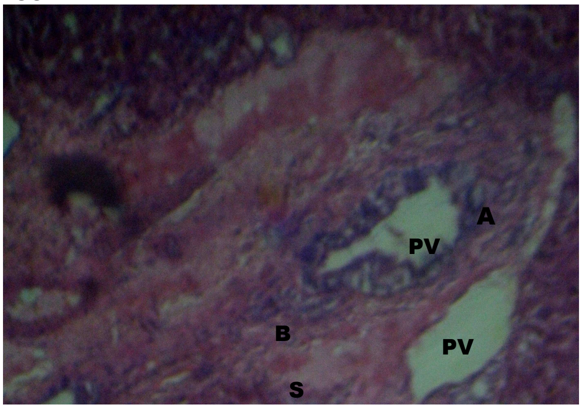

Figure 3: Transverse section of squirrel liver from Obukpa, showing the hepatic portal vein PV, hepatic artery A, bile ductulus B and hepatic sinusoids S. H&E x 100

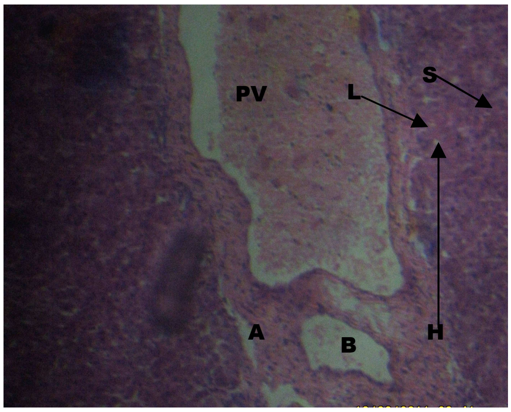

Figure 4: Transverse section of squirrel liver from Ibagwa showing the hepatic portal vein PV, bile ductulus B, hepatic artery A, plates of hepatocytes H, hepatic sinusoids S and lymphocytes L. H&E x 100

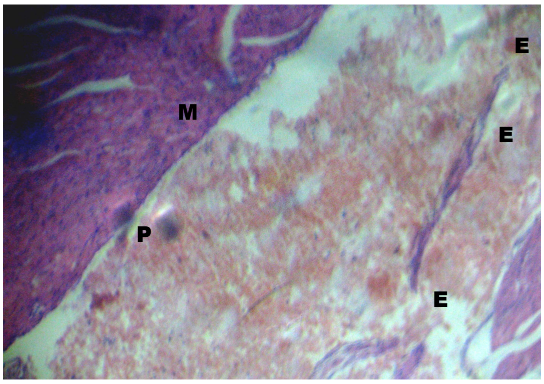

Figure 5: Transverse section of squirrel heart from Ede-Oballa, showing the tunica media M, the flattened cells pericytes P and endothelial cells E. H&E x 100

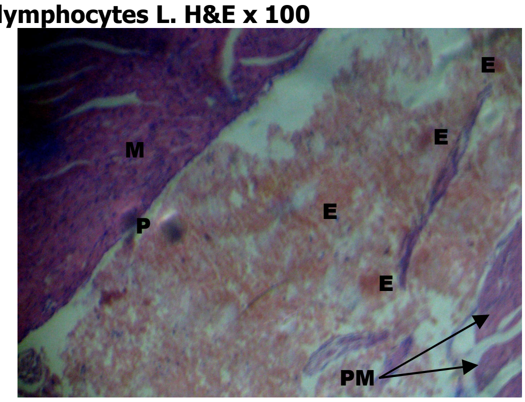

Figure 6: Transverse section of squirrel heart from Obukpa, showing endothelial cells E, the Tunica media M, flattened cells pericytes P, papillary muscles PM of the ventricle. H&E x 100

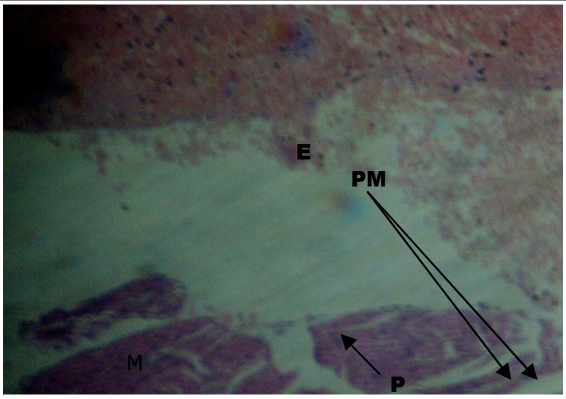

Figure 7: Transverse section of squirrel heart from Ibagwa, showing the papillary muscles PM, the tunica media M, flattened cells pericytes P. H&E x 100

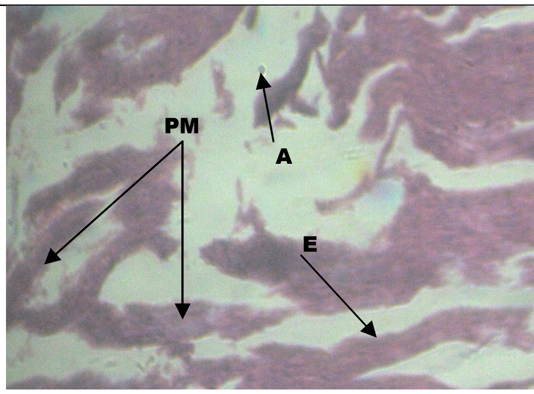

Figure 8: Transverse section of squirrel heart from Ede-Oballa, showing the papillary muscles PM, the endocardium (tunica intima), branch of coronary artery A. H&E x 100

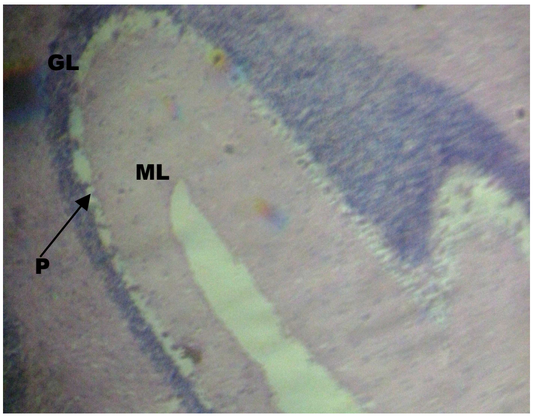

Figure 9: Transverse section of squirrel brain from Eha-Alumona, showing the outer molecular layer ML, an inner granular layer GL, and purkinje cell of the cerebellar cortex. H&E x 100

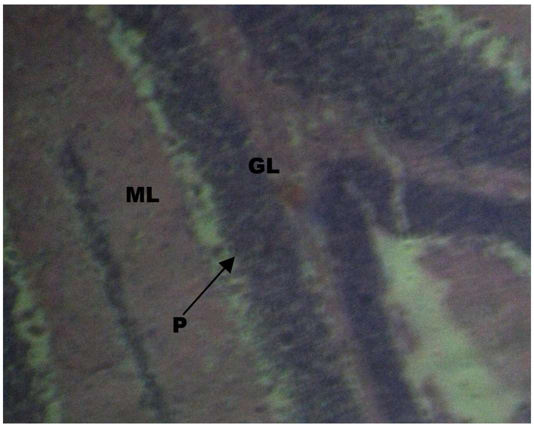

Figure 10: Transverse section of squirrel brain from Ibagwa, showing the outer molecular layer ML, an inner granular layer GL, and purkinje cell of the cerebellar cortex. H&E x 100

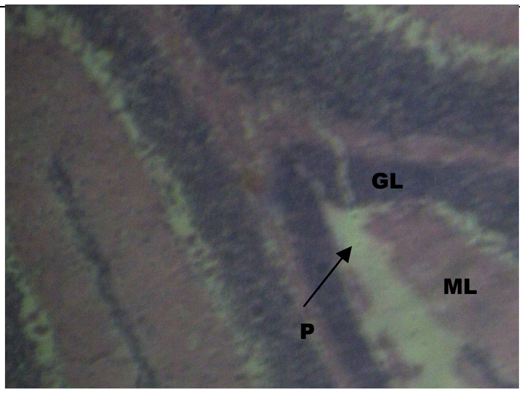

Figure 11: Transverse section of squirrel brain from Obukpa, showing the outer molecular layer ML, an inner granular layer GL, and purkinje cell of the cerebellar cortex. H&E x 100

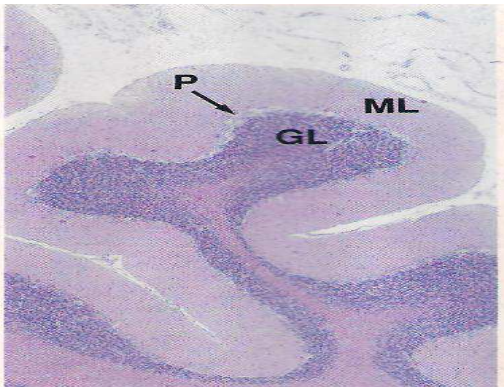

Figure 12: Transverse section of squirrel brain from Ede-Oballa, showing the outer molecular layer ML, an inner granular layer GL, and Purkinje cell of the cerebellar cortex. H&E x 100

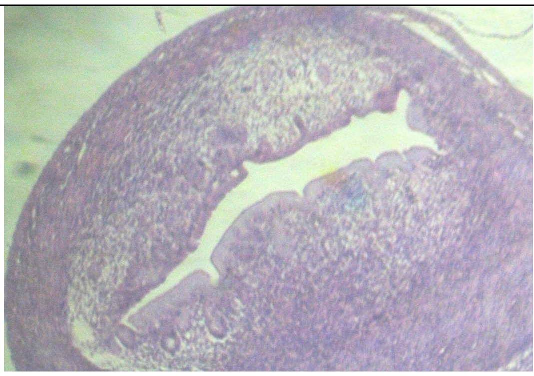

Figure 13: Transverse section of squirrel testis from Eha-alumona H&E x 100

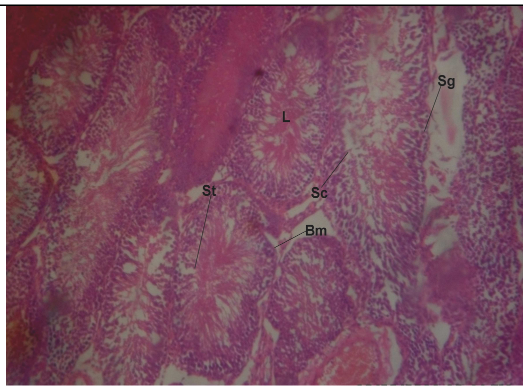

Figure 14: Transverse section of seminiferous tubule of squirrel testis from Eha-alumona, showing basement membrane BM, spermatogonia Sg, spermatid St, lumen L. H&E x 100

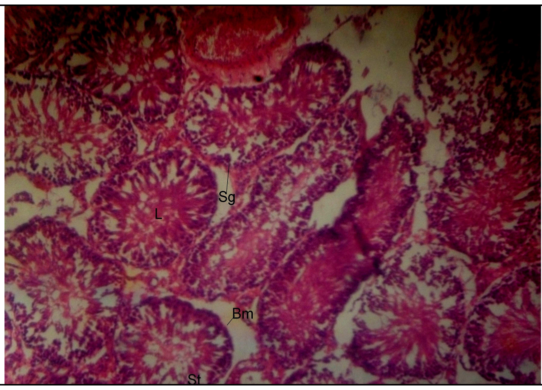

Figure 15: Transverse section of seminiferous tubule of squirrel testis from Obukpa, showing Basement membrane BM, spermatogonia Sg, spermatid St, lumen L. H&E x 100

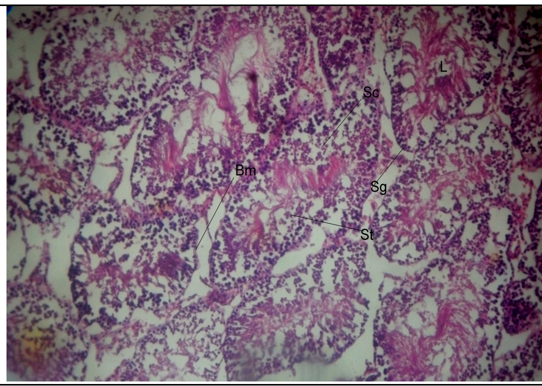

Figure 16: Transverse section of seminiferous tubule of squirrel testis from Ibagwa, showing basement membrane BM, spermatogonia Sg, spermatid St, lumen L. H&E x 100

Figure 17: Transverse section of Seminiferous tubule of Squirrel Testis from Ede-Oballa, showing basement membrane BM, spermatogonia Sg, spermatid St, lumen L. H&E x 100

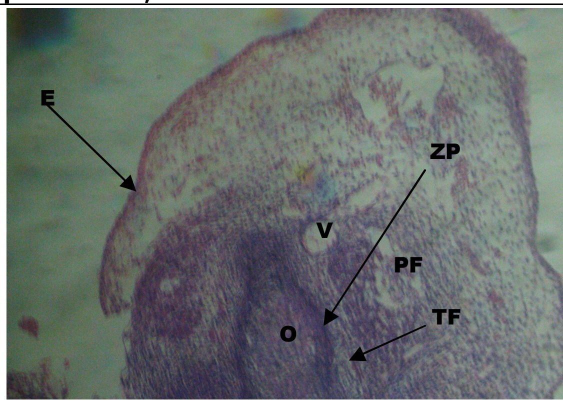

Figure 18: Transverse section of squirrel ovary from Ede-Oballa, showing the primordial follicles PF, zona pellucida ZP, oocyte O, a blood vessel V, theca folliculi TF, columnar epithelial cell E. H&E x 100

through the granular cell layer and an extensively branching dendrites system, which arborises into the outer molecular layer. There were no differences in the architecture of the brain tissue of squirrels sampled from the four villages in Nsukka agro-ecological zone (Figures 9 – 12).

Testis: A section of the seminiferous tubule consists of a basement membrane (BM) which serves as the tubule's membrane, mitotic spermatogonia (Sg) which undergo active mitosis to give rise to the primary spermatocytes (Sc). The primary spermatocytes undergo the first meiotic division to form the secondary spermatocytes. They in turn undergo a second meiotic division to form the spermatids (St) (Figures 13 – 16) which undergo maturation to form the spermatozoon. The newly formed spermatozoon is absorbed into the lumen of the tubule where they are drawn into the epididymal walls of the testis for temporary storage. There were no differences in the architecture of the seminiferous tubules of squirrels sampled from the four villages in Nsukka agro-ecological zone (Figures 13 – 16).

Ovary: The ovaries of all mammals have a similar basic structure. Their overall appearance, however, varies considerably in accordance with the species differences in ovarian cycle and the stage in the cycle at which the ovary was examined. A transverse section of the ovary showed a primordial follicles (PF), which is composed of primary oocytes (O) (Figures 17 and 18) surrounded by a single layer of flattened follicular cells. This PF exists in a mature ovary as undeveloped follicle. Also, the zona pellucida (ZP), a thick homogenous layer of glycoprotein and acid proteoglycans, develops between the Oocyte and follicular cells and serves as the eggs membrane. The theca follicle (TF) is an organized layer around the follicle and is separated from the granulose cells by a basement membrane. The epithelial cell (E) is found on the surface of the ovary. The blood vessel (V) also helps in the circulation of their blood. There were no differences in the architecture of the ovaries of squirrels sampled from the four villages in Nsukka agro-ecological zone (Figures 17 and 18).

DISCUSSION

In the present study, the squirrels' livers were normal and develops embryologically as outgrowth of the primitive gut. The hepatic portal tracts were normal and were in line with the report of Robert *et al*. (2004). Cullen and Marion (1996) reported that a few of the portal tracts had inflamed liver, the inflammatory infiltrate penetrated the limiting plate and extended into the adjacent parenchyma. Robert *et al*. (2004) reported centrilobular hepatocytic degeneration or necrosis occurred in the liver in the IP-infected animals. Inflammatory cell infiltration in the lobules was minimal. The hepatic portal veins the organ, from which absorbed food products pass directly from the gut to the liver. Oxygen required to support liver metabolism in squirrels is supplied through the hepatic artery and the hepatic vein, for venous drainage. The liver metabolizes glycogen, (gluconeogenesis), synthesizes albumin and clotting factors, helps in the destruction of spent red cells and reclamation of their constituents (Young and Heath, 2000). Lowenstine (2003) reported that hepatic hemosiderosis was a common finding in marmosets, tamarins, owl monkeys and other species of new world primates and in lemurs and gorillas. Hepatocellular iron deficiency may become severe enough to lead to alterations in hepatocellular function (hemochromatosis). The heart was normal without degenerations and constitutes the circulatory system of squirrels (Young and Heath, 2000). The cerebellar cortex forms a series of deeply convoluted folds or folia, supported by a branching central medulla (M), of white matter in the brain. Kunjan *et al*. (2006) reported that the Arctic ground squirrel (*S. parryii*) had eosinophilic cytoplasm, dark-staining triangular-shaped nucleus, and eosinophilic-staining nucleolus. The low partial pressure of oxygen (PO_2) levels found in AGS during euthermia is accompanied by increased hypoxia-inducing factor–1α protein levels in brain, suggesting that this species experiences mild, chronic hypoxia attributable to low respiratory drive. Kunjan *et al*. (2006) also reported that learning how the ground squirrel's brain recuperates could not only help scientists understand the brain's plasticity, but also suggest new ways to reverse or prevent cellular damage in neurodegenerative diseases. In this study, the testes showed a section of the seminiferous tubule and consist of a basement membrane (BM), which serves as the tubule's membrane. The newly formed spermatozoon, then, is absorbed into the lumen of the tubule, where they are drawn into the epididymal walls of the testis for temporary storage. This study was similar to the report of Hoque *et al.* (2011), that testicular parenchyma of ground squirrel is composed of seminiferous tubules (from which spermatozoa is formed) and leydig cells or interstitial cells. The sertoli cells are responsible for phagocytosis of residual cytoplasm cast off during maturation of spermatocytes and for synthesis of androgen-binding protein which is essential for proper germ cell differentiation and leydig cells secrete testosterone responsible for male sexuality (Young and Heath, 2000; Hoque *et al.,* 2011). Sever and Sengel (2006) reported that the spermathecal secretions may serve to attract and prolong the viability of sperm, but sperm that become enmeshed in the secretions or epithelium are phagocytized. They also reported that the bundles of sperm are aligned in parallel clusters and showed similar orientation. Even when sperm are not crowded into a tubule, sperm can be found with their nuclei embedded in the secretion matrix bathing the surface of the spermathecal epithelium. Abney and Keel (1989) reported damage to the germinal epithelium and resulting to infertility in humans and experimental animals as well as the degree of damage to the different stages of germ cell development. They also reported morphological alterations in Sertoli and Leydig cells in terms of cellular hypertrophy and hyperplasia. Shackelford and Goetz (2007) reported that in man as sperm cells mature they move between sertoli cells from the basal toward the adluminal compartment of the seminiferous tubule. Occluding junctions that interconnect adjacent sertoli cells shield secondary spermatocytes, spermatids and spermatozoa from autoimmune recognition. Nieschlag *et al*. (2006) reported that amphibians and most fish do not possess seminiferous tubules, instead the sperm are produced in spherical structures called sperm ampullae. Under a tough membranous shell, the tunica albuginea, the testis of amniotes and some teleost fish, contains very fine coiled tubes called seminiferous tubules. The developing sperm travel through the seminiferous tubules to the rete testis located in the mediastinum testis, to the efferent ducts, and then to the epididymis where newly created sperm cells mature. The sperm move into the vas deferens, and are eventually expelled through the urethra and out of the urethral orifice through muscular contractions. Nieschlag *et al*. (1989) *reported* that the testes of the non-boreotherian mammals such as the monotremes, armadillos, sloths, elephants remain within the abdomen. There are also some boreoeutherian mammals with internal testes, such as the rhinoceros. The ovaries of all mammals have a similar basic structure. In this study, a transverse section of the ovary showed a primordial follicles which is composed of a primary oocytes surrounded by a single layer of flattened follicular cells. This study is similar to the report of Young and Heath (2000). Walker *et al.* (2009) reported that in ovaries of aging squirrel monkeys (*Saimiri sciureus*), clusters of granulosa cells occur that resemble granulosa cell tumours in humans. These appear to be a normal change with age in this species. Elene *et al*. (2006) reported that the ovary of the squirrel monkey consist of a large almond-shaped structure with the lateral margin of the ovary covered by a simple cuboidal epithelium known as the germinal epithelium. However, the germinal epithelium actually is a continuation of the layer of cells that lines the peritoneal cavity.

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