HISTOLOGICAL AND HISTOCHEMICAL CHANGES OF THE FEMALE REPRODUCTIVE ORGANS OF GIANT AFRICAN LAND SNAILS (Archachatina marginata) WITH LENGTHS OF AESTIVATION

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ABSTRACT

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The histological and histochemical alterations through aestivation were determined in giant African land snails, Archachatina marginata. The experiment was conducted using 40 reproducitively matured snails, laid out in a completely randomized design, with 2 snails replicated four times. H and E staining was used to reveal the alterations in tissue general structures while Von Kossa staining techniques and Periodic Acid Schiff reaction were employed to demonstrate calcium and glycogen deposition respectively. The processed slides were examined with the aid of light microscope using a low power magnification (X200). Transverse section of the oviduct of Archachatina marginata as influenced by length of aestivation response conferred structural and content alterations to the tissues. The tissues were characterized by branched lumen lined with ciliated columnar epithelium. The basement membrane gained more prominence with underlying supportive tissue (ST) with lengths of aestivation. With respect to the albumin gland, vigorous pin-like cells were observed scattered all around the tissue at the 3rd week dormancy. The 6th week dormancy section displayed wider intercellular spaces. The vagina in its transverse section revealed tissues lined with ciliated columnar epithelium with prominent muscular wall. The lumen was prominent in the pre aestivated tissue but appeared collapsed during aestivation. Tissues of the oviduct showed no deposit of Calcium at the various lengths of aestivation investigated. Intracellular storage form of carbohydrate (glycogen) was moderately evident in the pre aestivated sections and accumulated even more at the 3rd week dormancy length. However, gross depletion was observed at the 6th week of aestivation. Summarily, aestivation resulted in slight disorientation of the tissue structure. The supporting tissue however, gained more prominence at the aestivated state probably for stability and to hold the cells in shape while dehydration last. Therefore, Archachatina marginata can withstand six-week aestivation period with no adverse effect to its reproductive histology.

Keywords: Aestivation, Archachatina marginata, Histology, Female reproductive organs, Calcium, Glycogen.

INTRODUCTION

The tropics is characterised with intermittent dry and wet periods. Since snails and other ectotherm lack physiological thermal control, free-living snails therefore withdraw into their shells by covering the shell aperture with a calcified mucous membrane called the epiphragm; for haemostatic stability and minimize water loss due to evaporation. In which case they aestivate by reducing mobility, reproductive behaviour and growth (Omoyakhi et al., 2008a,b). Water loss is further retarded by the use of discontinuous breathing pattern; the pneumostome opens intermittently to allow a rapid exchange of CO₂ and O₂ (Hermes-Lima et al., 1998) as a means of coping with the condition.

Series of systematic investigations was set out to provide adequate scientific information on the phenomenon - aestivation (Omoyakhi et al., 2008a,b; Omoyakhi and Osinowo, 2010a,b; Abdussamad et al., 2010; Ajayi et al., 2012). Among others, physiological implications of aestivation and arousal was monitored by taking measurable parameters such as weight, length and width of the male and female reproductive organs for assessing reproductive soundness and fertility during and post aestivation in A. marginata and A. achatina (Omoyakhi and Osinowo, 2011a, b, 2012). But how these are translated from the simpler form of life (Cells and tissues) has so far received the least attention in Giant African Land Snails (GALS). This study was therefore conducted to examine the changes in the tissues array and the histochemical dynamics of the female reproductive organs of Archachatina marginata under different lengths of aestivation.

MATERIALS AND METHODS

Location and experimental animals

The study was carried out at the University of Benin Teaching and Research Farm, University of Benin, Edo State, Nigeria. The farm is located within the tropical rain forest vegetation zone of Southern Nigeria lying between longitude 5°E and 6°42'E and latitude 5°45 and 7°34'N of the equator (NAA, 2013). On the north Edo is bounded by Kogi state, to the east is Anambra state, south by Delta and west by Ondo state. The climate of Edo is humid. The histological and histochemical procedures were carried out at the University of Benin Teaching Hospital, Benin City.
Experimental design and induction of aestivation
The experiment was conducted using 40 reproductively matured snails, laid out in a completely randomized design, with 2 snails replicated four times. Snails were induced to aestivate under prevailing atmospheric conditions in this study by discontinued moistening of soils and withdrawal of feed and water as reported in several researches on snail aestivation (Omoyakhi et al., 2008a, b; Ajayi et al., 2012; Adeoba et al., 2012).

Tissue collection and dissection procedures
Snail shells were cleaned thoroughly to remove the adhering fluid and dissected to obtain the female reproductive organs, according to the procedures outlined by Segun (1975).

Histological and histochemical examination
Pieces of organs were promptly and adequately fixed chemically as soon as the organs were detached using 10 % neutral buffered formolseline (Junqueira and Carneiro, 2003). With the aid of an automatic tissue processor (Zeiss Axiostop 40) the tissues were dehydrated and eventually cleared in two changes of xylene at room temperature. Further processing of the tissue followed the sequence of impregnation, embedding (using Leica EG1116), sectioning (with the aid of a microtome, Leica RM 2235). After which the sections were stained using Haematoxylin and Eosin (often abbreviated H & E) method. This was to reveal tissue general structure including degree of intercellular spacing, peripheral thickening or lesion, inflammation and cell size. Von Kossa staining techniques and Periodic Acid Schiff reaction were employed to demonstrate calcium and glycogen deposition respectively (Burkitt et al., 1999). Mounting and labeling followed closely. These so processed slides were examined with the aid of light microscope using a low power magnification (X200). The examined slides were then photomicrographed (TP 1020 Memmert) and presented as such.

RESULTS
The histological changes of the female reproductive organs of A. marginata are presented in figures 1 – 6. Each figure took account of the pre aestivated and aestivated micrographs at the respective length for each organ. Figure 1 – 4 demonstrate the general tissue structure and alterations as impacted by the lengths of aestivation. Figures 5 and 6 are micrographs of specially stained sections to demonstrate Calcium and glycogen deposition respectively. The obvious features in the micrographs are highlighted and defined alongside.

Demonstration of general tissue structure
Figure 1 shows a transverse section of the oviduct of Archachatina marginata as influenced by length of aestivation response. The treatments conferred structural and content alterations to the tissues. The tissues were characterized by branched lumen lined with ciliated columnar epithelium. The basement membrane gained more prominence with underlying supportive tissue (ST) with lengths of aestivation. The histological changes of aestivation lengths on the albumin gland of Archachatina marginata is presented in figure 2. Undefined segmentations of the tissues are observed at the pre aestivation (micrograph 0A) and the 3th (micrograph 3A) and 6th (micrograph 6A) week aestivation sections. However, vigorous pin-like cells were observed scattered all around the tissue at the 3th week dormancy. The 6th week dormancy section displayed wider intercellular spaces. As shown in Figure 3; the pre aestivated section (micrograph 0A) of the vagina of Archachatina marginata showed lumen prominently lined with ciliated columnar epithelium with outstanding muscular walls. These attributes diminished considerably at the 3th week aestivation and much more at the 6th week.

Histochemistry
Transverse sections of the tissue of oviduct of Archachatina marginata showed no deposit of Ca (Fig. 4) at the various lengths of aestivation investigated. Albumin gland tissues of Archachatina marginata were positive for glycogen deposition (deep red, traditionally describe as magenta) as demonstrated by PAS histochemical staining techniques. Intracellular storage form of carbohydrate (glycogen) was moderately evident in the pre aestivated sections (Figure 4, micrograph 0A) and accumulated even more at the 3th week dormancy length. However, gross depletion was observed at the 6th week of aestivation.

DISCUSSION AND CONCLUSION
Note worthy is the colour changes with lengths of aestivation in the Oviduct of Archachatina marginata resulting from the dynamics in tissue components. The folds are covered by a simple columnar, ciliated epithelium. Prominent is the tall columnar ciliated cells with the nucleus located towards the luminal aspect of the cells. They however, also contain non ciliated cells with basally located nuclei which has secretory function. These are probably for nutrition and protection of the ovum (Burkitt et al., 1999). The extensive branching of the lumen as observed in the pre aestivated tissues (figure 1 micrograph 0A) were also affected by the lengths of aestivation. Aestivation resulted in slight disorientation of the tissue structure. The supporting tissue gained more prominence at the aestivated state probably for stability and to hold the cells in shape while dehydration last. The condition was retained within the aestivation period as there was no much difference in the tissues at 3th and 6th week aestivation.
Within the study period, calcium deposit was not evident in the tissue of the oviduct. Although Tompa and Wilbur (1977) reported increasing calcium content of eggs with passage along the free oviduct in C. asperses, they also opined that calcium may be mobilized from elsewhere in the body and transported to the pallial gonoduct via the haemolymph.

Micrograph 0A (pre aestivated tissue) of the oviduct (Fig. 1) captured a clear sperm groove indicating non-conduction of sperm at this study period. The epithelia lining is of the pseudostratified epithelium which corroborate with those of human origin (Burkitt, et al., 1999). The muscular wall (arranged into inner and outer layers) increased in thickness with widening yet more organized tissue with lengths of aestivation. Lipid deposition was observed at the 3rd week dormancy state and was probably used up; been absent at the 6th week. Cling of cell mass and granules were also typical features of the 6th week aestivated tissue. Although Gomez (2001) reported that oviduct exhibit calcium-transporting tissues, the tissues of oviduct (figure 4) in this study were completely void of Ca at the various lengths of aestivation.

The prominent circular or pear shaped follicle was not observed in the pre aestivated tissue of albumen gland. A distorted tissue resulting in undifferentiated follicles characterized the 3 weeks aestivation treatment but with vigorous pin like cells scattered all around the tissue. The 6th week dormancy section was similar to the pre aestivated state in colour probably connected with stress from long seasonal activities for the pre aestivated tissue and dehydration length for the 6th week dormancy and perhaps also due to depletion of galactose in the albumen necessary for tissue maintenance. Besides, the albumen is only temporarily stored within the albumen gland.
Figure 5: Calcium deposition on the tissue of Oviduct of *Archachatina marginata* with lengths of aestivation. Von Kossa staining techniques, 200 X. 0A = Pre aestivation, 3A, 6A = 3 and 6 weeks aestivation respectively. The tissues of oviduct completely void of Ca at the various lengths of aestivation.

Figure 6: Glycogen deposition on the tissue of Albumen gland of *Archachatina marginata* with lengths of aestivation. Periodic Acid Schiff Reaction (PAS) staining technique, 200 X. 0A = Pre aestivation, 3A, 6A = 3 and 6 weeks aestivation respectively.

The vagina in its transverse section revealed tissues lined with ciliated columnar epithelium with prominent muscular wall. The lumen was prominent in the pre aestivated tissue but disappeared eventually. This may not be unconnected with the tendency for the wall to collapse at dormancy as do the human vagina. According to Burkitt *et al.* (1999) the vagina in the relax state, have its wall collapse to obliterate the lumen. 

Summarily, aestivation resulted in slight disorientation of the tissue structure. The supporting tissue however, gained more prominence probably for stability and to hold the cells in shape while dehydration last. It can be concluded therefore that, *Archachatina marginata* can withstand six-week aestivation period with no adverse effect to its reproductive histology.
REFERENCES


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