Tropical Biomedicine 32(3): 497–503 (2015)

Histoarchitecture of ovary of *Haemaphysalis bispinosa* during engorgement period

Sreelekha, K.P.[1], Chandrasekhar, L.[2], Jyothimol, G.[3], Lenka, D.R.[3], Aswathi, V.[1], Adarshkrishna, T.P.[1], Ajithkumar, K.G.[3], Nair, S.N.[1], Ravindran, R.[3*], Juliet, S.[1] and Ghosh, S.[4]

[1]Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Pookode, Lakkidi, P.O., Wayanad, Kerala-673576, India

[2]Department of Veterinary Anatomy, College of Veterinary and Animal Sciences, Pookode, Lakkidi, P.O., Wayanad, Kerala-673576, India

[3]Department of Veterinary Parasitology, College of Veterinary and Animal Sciences, Pookode, Lakkidi, P.O., Wayanad, Kerala-673576, India

[4]Division of Parasitology, Indian Veterinary Research Institute, Izatnagar, UP- 243122, India

[*]Corresponding author email: drreghuravi@yahoo.com

Received 27 July 2014; received in revised form 23 December 2014; accepted 26 December 2014

Abstract. The ovary of *Haemaphysalis bispinosa* was of panoistic type with asynchronous development of oocytes. The wall of the ovary was composed of a layer of epithelial cells to which the oocytes were attached by means of pedicel cells with elongated nucleus. The oocytes were classified into stages I to V based on morphologic characteristics like size and shape, presence / absence of germ vesicle, cytoplasmic appearance, presence or absence of yolk granules and presence of chorion. Day wise changes were in the form of occurrence of oogonia from partially fed upto day zero of engorgement, presence of all stages of oocytes on day one and two after engorgement and onset of degenerative changes in oocytes from day three onwards. Degeneration was complete on day eight with the appearance of polymorphism, vacuolation, cytoplasmic blebbing and autophagic activity in oocytes.

INTRODUCTION

Ticks and tick borne diseases cause huge financial loss in dairy as well as leather industry. They are the most important vectors of disease causing pathogens in domestic and wild animals (Antunes *et al.*, 2012). In cattle industry, the average tick burden causes an annual weight loss of 0.7 kg/tick (Hakim *et al.*, 2007). More than 80 per cent of world cattle population is exposed to tick infestation causing a global annual loss of US$ 7000 million (FAO, 1984). In India, the cost of TTBD control in animals was estimated as US $ 498.7 million per annum (Minjauw & McLeod, 2003).

The most common tick species under the genus *Haemaphysalis* in the oriental region is *Haemaphysalis bispinosa* and it belongs to the family Ixodidae. They are reported in domestic cattle, buffalo, horse, goat, sheep, wild mammals, rodents and several bird species from many states of India (Geevarghese *et al.*, 1997). This species acts as vector for *Babesia motasi* and *B. ovis* in sheep and goat, *B. equi* in horses and donkeys and *B. canis* and *B. gibsoni* in dogs (Taylor *et al.*, 2007).

Tick control depends largely on the use of different chemical acaricides. The development of resistance against commonly available acaricides poses a huge problem in tick control. Hence, new classes of acaricides with different modes of actions are desperately needed for the cattle industry to thrive. Reproductive potential in ticks is very high and drugs targeting this system can effectively control the parasite population. Detailed studies on the normal histology of the reproductive organs of *H. bispinosa* will

immensely help in understanding the changes that occur in their ovaries when newer drugs targeted to reproductive system are developed and used against these ticks.

No information is currently available on the normal structure of ovaries of *H. bispinosa*. Therefore, the present work aims to contribute data on the histological aspects of the ovaries of *H. bispinosa.*

MATERIALS AND METHODS

Ticks

Partially fed (4-5 days prior to complete engorgement) and fully engorged females of *H. bispinosa* were used in this study. Six partially fed ticks were immediately dissected. Engorged females of *H. bispinosa* (Forty two specimen) were collected from infested animals and maintained in the Biological Oxygen Demand incubator (BOD) (28 ± 1ºC and 85 per cent relative humidity). Six fully engorged female ticks were taken out from BOD incubator on the day of engorgement (day zero) as well as first, second, third, fifth and eighth days after engorgement. The ovaries were dissected out in 1.2 per cent saline using stereozoom microscope (Edward *et al.*, 2009).

Histology

Ovaries were fixed in formaldehyde acetone fixative in the ratio of 9:1 for 18 hours at 4°C (Arnosti *et al.*, 2011). Dehydration was carried out in ascending grades of ethanol for 15 minutes each followed by clearing in xylene for 20 minutes. They were embedded in paraffin (melting point 58-60°C). Serial sections were cut at 4 µm thickness, stained by hematoxylin and eosin staining method (Singh & Sulochana, 1996) and observed under microscope (Leica Germany).

The oocytes were classified based on size and shape, presence / absence of germ vesicle, cytoplasmic appearance, presence or absence of yolk granules and presence of chorion (Denardi *et al.*, 2004; Saito *et al.*, 2005; Oliveira *et al.*, 2006; Sanches *et al.*, 2010).

RESULTS

The ovary of *Haemaphysalis bispinosa* consisted of a single tubular horseshoe shaped continuous structure located at the posterior third of the tick's body.

The ovary of adult tick consisted of central lumen lined by small epithelial cells and oocytes in different developmental stages. Two poles could be identified on both the ends of the lumen. The oocytes were attached to the ovary wall by specialized epithelial cells called pedicel cells (Fig. 1H) with elongated nuclei. Pedicel cells of mature oocytes were parallelly arranged.

The oocytes could be classified into stages varying from I to V (Table 1).

Day wise changes

Partially fed

Ovary of partially fed *H. bispinosa* revealed dense eosinophilic oogonia interconnected among themselves (Fig. 1F). Oocytes were not distinguished at this stage.

Day zero of engorgement

On the day of full engorgement, *H. bispinosa* ovary revealed dense eosinophilic oogonia interconnected among themselves and germinal epithelium at small locus towards one pole. Oogonia were clearly distinguished as spindle shaped masses having cord like connections radiating from two or more different sites. However, large numbers of refractile bodies of different sizes were seen attached to the connecting cords of oogonia. Very few mature oocytes were observed. Nurse cells were not observed.

Day one after engorgement

On day one after engorgement, a distinct arrangement of oocytes was observed with immature oocytes at one pole and more advanced stages towards the other pole. The maturing oocytes were attached to the ovary and showed a sequential arrangement starting from stage I to stage V in cross section. However, different stages of oocytes were noticed throughout the length of the

Table 1. Classification of oocytes of *H. bispinosa*

Oocyte I (Fig 1A)	Oocyte II (Fig 1B)	Oocyte III (Fig 1C)	Oocyte IV (Fig 1D)	Oocyte V (Fig 1E)
0.02-0.04 mm rounded and elliptical	0.06 – 0.09 mm elliptical	0.120 – 0.177 mm elliptical	0.190-0.205 mm round	0.210 – 0.340 mm round
Germ vesicle present	Germ vesicle present and basophilic	Germ vesicle located at pole facing pedicel basophilic	Germ vesicle not visible	Germ vesicle not visible
Homogenous cytoplasm	Homogenous cytoplasm	Fine granulation throughout cytoplasm	Coarse granulation	Coarse granulation
Yolk droplets not formed	Yolk droplets not formed	Yolk droplets not fully formed	Yolk droplets fully formed	Yolk droplets merged in the centre
Chorion absent	Chorion absent	Thin chorion	Thick chorion	Thick chorion
Dominant on day zero and day one after engorgement	Dominant on the day one and two after engorgement	Dominant on day two and three after engorgement	Dominant on days three to five after engorgement (egg laying time)	Dominant on day five (egg laying time)

ovarian tube. The first stage oocytes showed strong basophilic reaction and high nuclear to cytoplasmic ratio was observed in the germinal epithelium.

Day two after engorgement

Most of the oocytes were in stage II and III and reflected an intense eosinophilic reaction. Nuclear to cytoplasmic ratio of germinal epithelium decreased comparatively indicating maturation of cells.

Day three after engorgement

Eosinophilic reaction of oocytes was as strong as day two. Few degenerating oocytes were also appreciated (Fig. 1I). Some oocytes appeared polymorphic with the onset of a glassy appearance. A population of oocytes revealed conspicuous cytoplasmic activity (Fig. 1G).

Day five after engorgement

The ticks began oviposition. Ovary revealed increased numbers of polymorphic oocytes with glassy appearance (Fig. 1J). There were vacant spaces towards the periphery of stage V oocytes in which trafficking of small pinocytic vesicles were seen. Dense materials were seen near the periphery of oocytes. Peripheral yolk droplets showed autophagic activity which appeared as dense particles with vacuoles around them. Some oocytes showed autophagic activity throughout the cytoplasm.

Day eight after engorgement

All the oocytes revealed polymorphism and glassy appearance. Oocytes showed cytoplasmic blebbing (Fig. 1K) suggestive of programmed cell death.

Day nine after engorgement

In general, small oocytes appeared polymorphic and glassy while large oocytes were intense eosnophilic with dense dark granules around each lipid droplets.

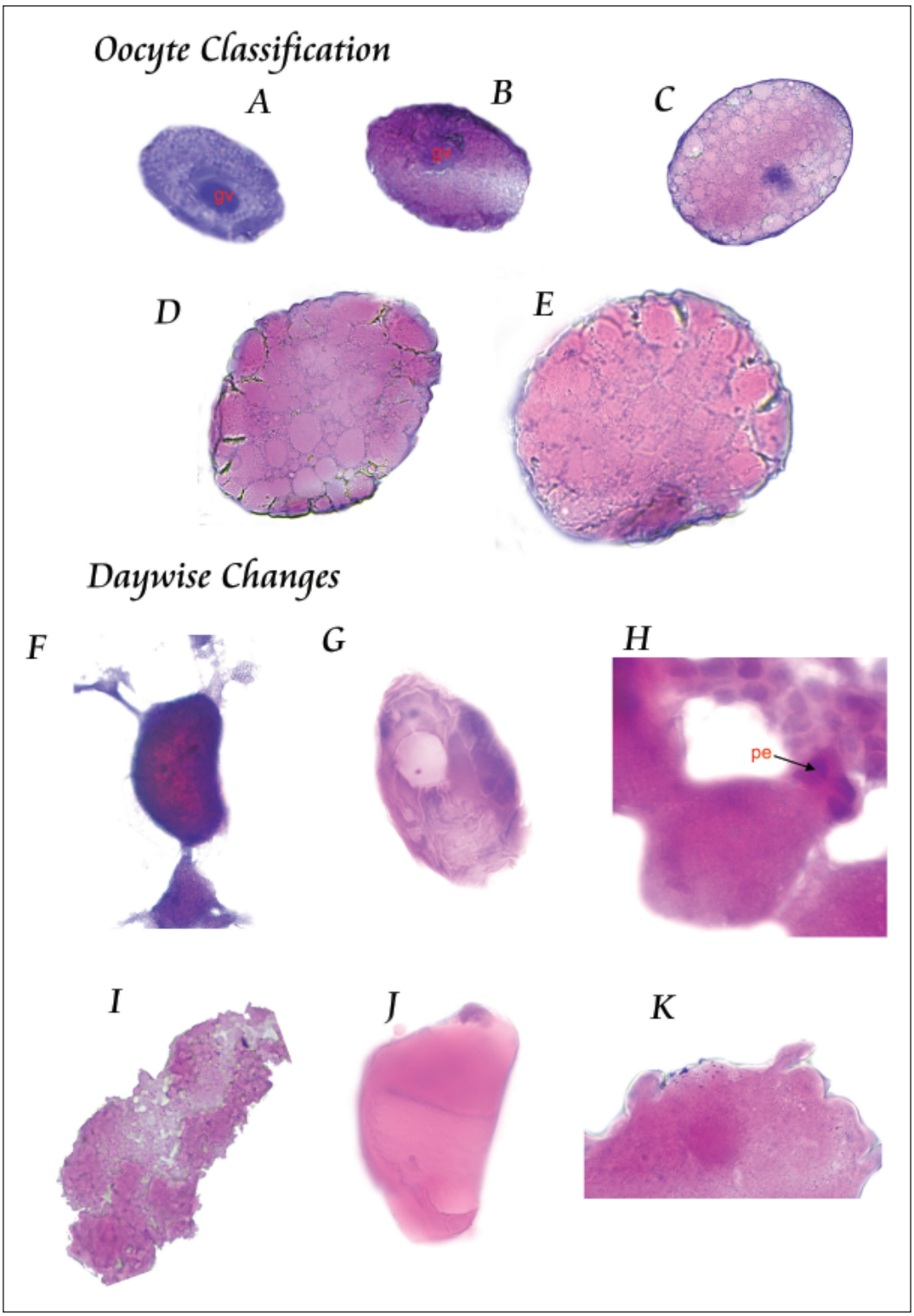

Figure 1. A) Oocyte stage I B) Oocyte stage II C) Oocyte stage III D) Oocyte stage IV E) Oocyte stage V F) Oogonia G) Oocyte showing conspicuous cytoplasmic activity H) Pedicel cells connecting the oocyte to the epithelium I) Degenerating oocyte J) Polymorphic oocyte K) cytoplasmic blebbing over the surface of oocyte; gv- germinal vesicle, pe- pedicel cell Bars: A-E – 20x, F-K- 100x

DISCUSSION

The ovary of *H. bispinosa* consisted of single tubular horseshoe shaped structure located at the posterior third of the tick's body. Similar observations were made for the ovary of *Dermacentor andersoni* and *D. variabilis* (Sonenshine, 1994), *Amblyomma cajennense* (Denardi *et al.*, 2004), *A. braziliense* (Sanches *et al.*, 2010) and *R. sanguineus (Oliveira et al., 2005)*.

The ovary of *H. bispinosa* showed oocytes in various stages of development throughout the period of study. Oocytes in early stages of development were seen concentrated towards one pole of lumen while more mature oocytes were seen on the other pole. Hence, an asynchronous development of oocytes was observed in the *H. bispinosa* ovary. In other tick ovaries, the oogenesis was processed distal-proximally (Denardi *et al.*, 2004; Oliviera *et al.*, 2005). The present study demonstrated similar disposition of oocytes in the ovary of *H. bispinosa*.

The oocytes were attached to the germinal epithelium by a cluster of cells called pedicel cell in *R. appendiculatus* (Till, 1961). Similar observations were made for other species too (Denardi *et al.*, 2004; Oliveira *et al.*, 2005; Saito *et al.*, 2005). In the present study, these pedicel cells were identified as a bunch of cells at the base of oocytes, having elongated nucleus.

The ovary of *H. bispinosa* was identified as that of panoistic type since nurse cells were absent. Previously, panoistic type of ovary was detected in *A. cajennense* (Denardi *et al.*, 2004), *R. sanguineus* (Oliveira *et al.*, 2005), *B. microplus* (Saito *et al.*, 2005) and *A. braziliense* (Sanches *et al.*, 2010). In panoistic type ovaries, oogonia give rise to oocytes (Eberhard, 2003).

Based on histological studies of *H. bispinosa*, oocytes were classified into five stages on the basis of size, shape, presence or absence of germ vesicle, cytoplasmic appearance, presence of yolk granules and chorion (Denardi *et al.*, 2004; Oliveira *et al.*, 2005; Saito *et al.*, 2005; Sanches *et al.*, 2010). Distinct basophilic nature of stage I oocytes reported in the present study was not previously reported in literature. A clear visible nucleolus in stage I oocytes was also observed in this study as reported by Oliveira *et al.* (2005). Oocytes of stages (II – V) were similar to the previously reported stages of oocytes of *R. sanguineus* (Oliveira *et al.*, 2005).

Oliviera *et al.* (2005) reported that the cytoplasm of stage III oocytes was full of yolk granules of various sizes of which the smaller grains occupying the central region while larger ones in the surroundings. This observation was not appreciated in *H. bispinosa*. Similarly, Oliviera *et al.* (2005) did not describe the merging of yolk droplets in the centre of stage V oocytes of *R. sanguineus* which was observed in the present study.

On the day of full engorgement, *H. bispinosa* ovary revealed dense eosinophilic spindle shaped oogonia and large numbers of refractile bodies of different sizes attached to the connecting cords of oogonia. It is presumed that these refractile bodies may be lipid droplets incorporating into the oocytes after a blood meal. According to Georgia *et al.*, 2005, in insects the digested blood contains a large amount of lipids which is secreted to the hemolymph and taken up by the growing oocytes for producing molecules such as vitellogenins (VGs).

The ovary of *H. bispinosa* on day one after engorgement showed oocytes in various stage of development confirming that vitellogenesis started on day one itself. The high nuclear to cytoplasmic ratio of germinal epithelium observed on day one could be due to the active synthesis processes occurring in the young epithelial cells and the strong basophilic reaction of stage I oocytes could be attributed to increased ribosomal content (Diehl & Aeschlimann, 1982).

Increased eosinophilia of oocytes on day two after engorgement was previously observed for *R. (B.) annulatus* (Kady *et al.*, 2001). The germinal epithelium showed marked reduction in basophillia. Hence, it was concluded that multiplication of germinal epithelium mainly occurred on the

day zero of engorgement and day one after engorgement while maturation of cells occurred later.

On day three after engorgement, stage two oocytes of *H. bispinosa* ovary revealed conspicuous cytoplasmic activity. Saito *et al.* (2005) also reported increase in the number of organelles in oocytes at the end of stage two and beginning of stage three. Onset of degenerative changes in the form of polymorphism of oocytes and the appearance of glassy cytoplasm were appreciated at this stage. Also, few oocytes showing typical characteristics of stage six oocytes described by Saito *et al.* (2005) were also appreciated. Since such oocytes showed many degenerative changes, the authors preferred to include such oocytes under degenerative changes rather than stage VI in the present study.

Egg laying started in *H. bispinosa* on the fifth day after engorgement. The stage V oocytes on the fifth day showed intense trafficking of pinocytic vesicles towards the basal membrane. Together with this, degenerative changes in the form of polymorphic and glassy oocytes and autophagic activity within the yolk droplets were seen.

On day eight after engorgement, cytoplasmic blebbing and abnormal morphology of nucleus were observed along with various degenerative changes. Description of apoptotic cells concurred with previous studies (Wyllie & Duvall, 1992; Machacaka & Compton, 1993).

On day nine after engorgement, ovary of *H. bispinosa* presented the picture of complete degenerative changes of oocytes and germinal epithelium.

Thus progressive degenerative changes were seen in normal *H. bispinosa* ovary from day three onwards which peaked by day eight with the appearance of polymorphism, vacuolation, cytoplasmic blebbing and autophagic activity in oocytes. So normal degenerative process of tick ovaries has to be taken into account while studying effects of acaricidal agents on tick ovaries.

Acknowledgements. Financial supports from Indian Council of Agricultural Research – National Fund for Basic Strategic and Frontier Application Research in Agriculture "Chemo-profiling of potential phyto-acaricides and their functional characterization for controlling resistant cattle ticks (NFBSFARA/BSA-4004/2013-14)" and state plan project of Kerala Veterinary and Animal Sciences University "Establishment of state of the art diagnostic, training and demonstration facilities in the Department of Veterinary Parasitology, College of Veterinary and Animal Sciences, Pookode (RSB 2012023)" are thankfully acknowledged.

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