Institute of Experimental Morphology and Anthropology

Acta morphologica et anthropologica, 5
Sofia•2000

Histomorphology of the female reproductive system of nymph and adult *Boophilus microplus* Canestrini (Acari, Ixodidae)

A. Saha, G. Chel, D. Ghosh

Department of Life Science, Tripura University, Agartala, India

The female reproductive system of *Boophilus microplus* studied under light microscope was observed to possess a "U" - shaped organization with numerous oocytes growing around the tubular oviduct. No special nurse cells were observed and the growing oocytes were connected to the oviduct epithelium by funicle cells. The growing oocyte had a large nucleus (germinal vesicle) possessing several nucleoli. The staining property of nucleolus revealed presence of two zones in it.

Key words: oogenesis, ovary, oocytes, Acari.

Introduction

The Cattle-tick *Boophilus microplus* is an economically damaging bovine ectoparasite [4] and this hard tick is distributed in different parts of the world including America, Australia, Asia, Africa [7]. Biological significance of tick has become important since it is the vector of Babesia, Anaplasma and Spirochaet [2]. The knowledge of anatomy and cytology of female reproductive organ of ixodid tick at various developmental stages is important because *Boophilus* transmits infections agents to their progeny through the eggs [1].

Brinton and Oliver [1] have elucidated the female reproductive physiology of *Dermacentor andersoni.* Yano et al. [8] have made ultrastructural studies of oogenesis of *Haemaphysalis longicornis* and El-Said [6] has worked out the histology of female reproductive organ of *Ambliomma cajennense*. But so far no work has been done on any aspect of oogenesis or reproductive system of the Indian Cattle-tick *Boophilus.* In view of that the present work communicates the gross anatomy and histology of female reproductive system and oogenesis of *B. microplus.*

Material and Methods

B. Microplus used in this study were collected from the body-coat of cattle in and around Agartala city and were brought to laboratory in small plastic jars. Nymphs and adults were kept separately. For light microscopy tick were dissected in normal saline at pH 7.2. The ovarian tissue was immersed in 'Susa' fixative [5] for 18 to 24 hrs and dehydrated in graded ethanol followed by paraffin infiltration and embedding at 58 °C. Several sections of 5 to 7 µm were cut by rotatory microtome, stained with Haematoxylin-Eosin or Bromphenol blue and observed under microscope. The ovarian tissue was processed for SEM investigation by routine method.

The dissected female reproductive organ of *B. microplus* were drawn to scale and measurements were made with adjustable ocular micrometer.

Results

Ovary of unfed nymph was tubular "U"-shaped structure covering up to posterior third of the body cavity. The paired oviducts originate near the female genital aperture and became connected to the terminal end of the ovary. Near the terminal end of joining of the paired oviduct lies the spermatheca at the antero-ventral margin of the body. Distinct lobes of accessory glands were noticed on the antero-lateral wall of the spermatheca, which was connected by the oviducts with each end of the ovary (Fig. 1). At this stage the length of the ovary varied from 2.5 mm to 5.5 mm and the ovarian diameter midway along its length was found to be ranging from 60 to 150 µm. However, the ovary became enlarged considerably during nymphal engorgement.

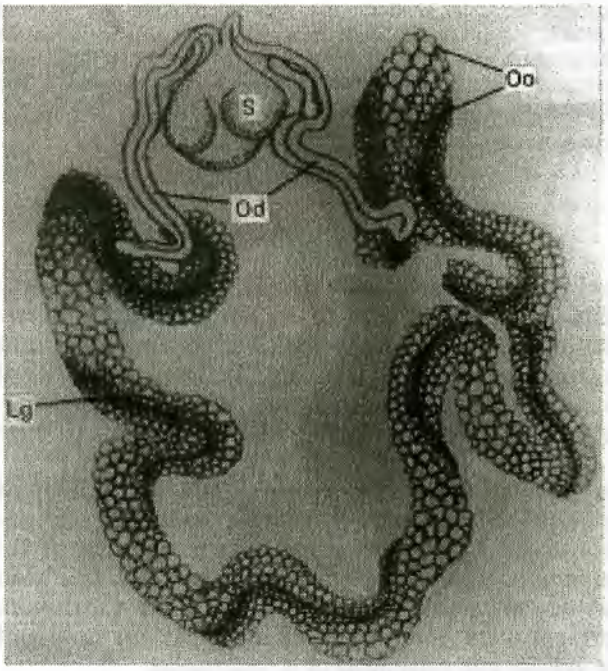


Fig. 1. Semidiagrammatic presentation of convoluted ovary of *Boophilus microplus* with oviduct (Od); spermatheca (S); oocytes (Oo) and longitudinal groove (Lg)

Ovaries in engorged adult female of *B. microplus* showed distinct changes in size and form. Ovary became about 7 mm in length and about 160 µm in width. The developed and enlarged oocytes (dia 59.2) protruded in the haemacoel from the surface of ovary. The oocytes were attached to the ovarian wall by cellular bridge, and longitudinal groove was radially apparent all along the long axis of the ovary (Fig. 2).

Histology of immature tick-ovary revealed the presence of formative oocytes embedded in follicular epi-

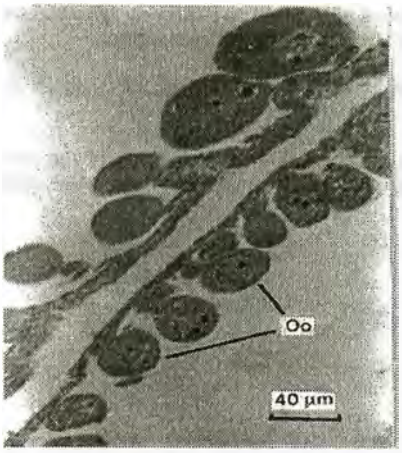


Fig. 2. Light micrograph of longitudinal section of the ovary of *B. microplus* with oocytes (Oo)

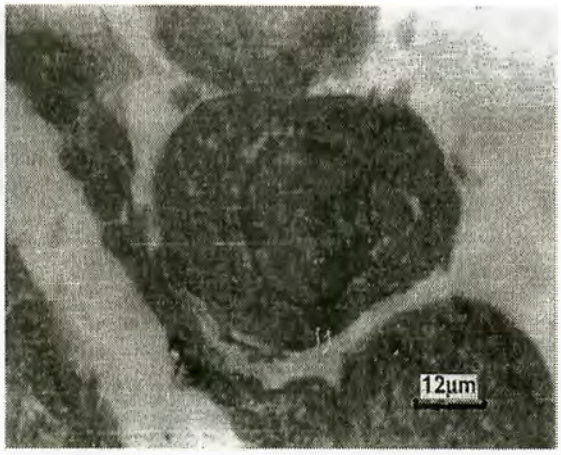


Fig. 3. Light micrograph of cross-section of oocytes showing the pedicle and well defined nucleus with nucleolus

thelium attached within the wall of the oviduct. Along with growth, the histological picture changed and in adult ovary the oocytes at various stages of growth were seen to be attached to the ovarian wall (Fig. 2). At this stage the diameter of the oocytes varied from 20 to 56 µm. Those were attached by cellular pedicle to the ovarian wall. The height of cellular pedicle was 16-28 µm. The oocyte nucleus with diameter 25 µm contained 2-4 well-defined nucleoli (Fig. 3, 4). The staining property of the nucleolus showed that its core was faintly stained while its margin was deeply stained (Fig. 4). The ovarian interstitial cells also possessed prominent nucleus with diameter about 6 µm. Sometimes their nucleoli were seen to be attached to the nuclear membrane. In early developing follicle the follicle cells were flat type and connective tissue elements held the oocyte with the ovarian wall.

Initially the egg cells grew in size and the cytoplasm remained finely granular. But within few days the cytoplasm became granular and drops of yolks were seen within the egg (Fig. 3, 4). These yolk droplets showed strong eosinophilia and also became intensely stained with bromphenol blue indicating their proteinacious nature.

Along with the growth of the tick, the developing egg cells bulged out and gave the appearance of grape-bunch (Fig. 5). The SEM picture of mature ovary showed the presence of the opercular side of egg towards the haemocoel side of the body cavity. At this stage the egg cells became more that 100 µm in diameter.

The oviducts and the common oviduct were composed of deeply staining columnar epithelial cells (Fig. 6). The cells were arranged over a thick basement membrane and the elliptical nuclei were centrally placed. The diameter of nuclei was about 4 µm. Near the vaginal end the stainability of the epithelium decreased. The lumen of the oviduct was seen to be filled with some secretion.

Discussion

On the basis of the results obtained in the present study it is apparent that structurally the female reproductive system of *Boophilus* possesses a simple organization but

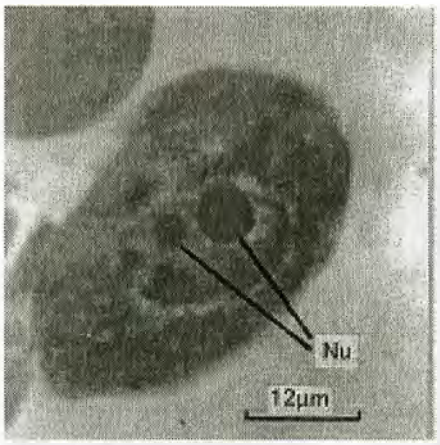
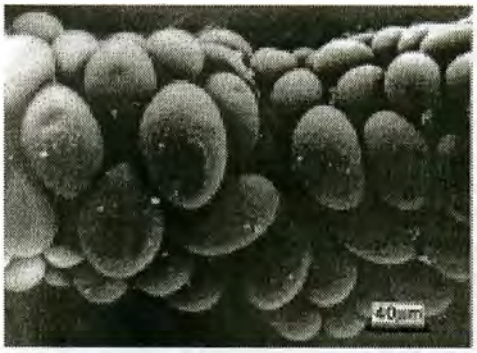
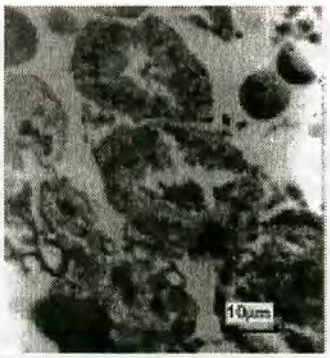


Fig. 4. Sectional view of oocyte showing the nucleus with distinct nucleoli (Nu) with two regions

Fig. 5. Scanning electron micrograph of ovary of *B. microplus*

Fig. 6. Light micrograph of cross-section of the oviduct showing deeply stained columnar epithelial cells





functionally very efficient since within a month a female tick laid about 1300-1400 eggs. In the present study a longitudinal groove was seen in the ovary, which was reported earlier by Brinton and Oliver [1] in Dermacentor. It appeared from the present study that there was no separate germarium and vitellarium with trophocytes and follicular cells but other features were almost similar with those of insects. The present study clearly indicated that the tissue of the ovarian wall worked as the germarium as seen in other ixodids [1]. The tick after engorgement underwent rapid growth and this happened due to the growth of the oocytes and vitellogenesis. The said feature had already been shown by Yano et al. [8], Brinton and Oliver [1] in *Dermacentor* and *Hyaloma.*

The staining property of the oocyte nucleoli observed in the present study was significant. Such features had already been reported in insects. In euckariotic cells ultrastructural studies had revealed the presence of two zones in the nucleolus — the granular and fibrilar zone [3]. It may be inferred that the observed staining property was related with that kind of organization of the nucleolus and it also seems that high amount of ribosomal RNA was synthesized in this structure during oogenesis.

Acknowledgements: The authors are thankful to the authority of Tripura University for providing necessary laboratory facilities and staff of RSIC, Shillong for technical help.

References

1. Brinton, L. P., J. H. Oliver. Gross anatomical, histological and cytological aspects of ovarian development in *Dermacentor andersoni* Stiles (Acari:Ixodidae). — J. Parasitol., **57**, 1971, No 4, 708-719.
2. Choudhuri, R. P. Insect parasites of livestock and their control. — Indian Council of Agricultural Research (New Delhi), Research Series, **29**, 1962, p. 43.
3. De Robertis, E. D. P., E. M. F. De Robertis. — In: Cell and Molecular Biology, Philadelphia, Holt-Saunders Int. Comp., 1980.
4. Gee, G. F. The economic importance of cattle tick in Australia, Cambera, Bureau of agricultural economics, 1959.
5. Gray, P. The Microtomist formulary and Guide, New York, McGraw Hill Book Comp. 1954.
6. EL-Said, A. A contriburion to the anatomy and histology of the female reproductive system of *Ambliomma cajennense* (Acarina: Ixodidae). — J. Egiptian Soc Parasitol., **22**, 1992, No 2, 391-400.
7. Sinha, S. R. P., B. N. Sinha, M. Z. Ansari, B. N. Sahai. Incidence of tick infection in Cattle, Buffaloes and Goats of Ranchi, Chotanagpur. — R. A. U. J. Res., **2**, 1981, No 3, 4, 37-41.
8. Yano, Y., T. Mori, S. Shirashi, T. A. Uchida. Ultrastructure of oogenesis in the Adult Cattle Tick *Haemophysalis longicornis*. — J. Fac. Agr. Kyushu Univ., **34**, 1989, No 1, 2, 53-67.