HISTOLOGICAL STUDIES ON EGG OF AAK GRASSHOPPER, *POEKILOCERUS PICTUS* FAB. (ORTHOPTERA : ACRIDIDAE)

Somenath Dey [16]

Abstract

This study has been undertaken to investigate the histology of egg of Poekilocerus pictus Fabricius under light and transmission electron microscope. The general histology of the egg largely resemble to those of other acridids, however, there are some unusual features.

Key words- *Poekilocerus pictus*, *Histology, Ovary, Oocyte, LM, TEM*

INTRODUCTION:

Poekilocerus pictus (Aak or painted grasshopper) common throughout the planes of India (Raziuddin and Anwar, 1997) is primarily a defoliator of Akand or Aak plants (*Calotropis procera*). This insect is a prolific breeder and besides *C. procera*, it feeds upon a number of alternative host plants, many of which are of economic value (Pruthi and Nigam, 1939; Pruthi, 1954; Parihar, 1974; Khurana, 1975; Raziuddin *et al*., 1991).

The insect ovary are consisting of many ovarioles which are in many cases, including Orthoptera are connected with the lateral oviduct in linear sequence (Wigglesworth, 1965; Chapman, 2000; Tembhare, 2006; Jackson *et al*., 2010). Insect eggs are covered externally by a complex multilayer egg shell consisting basically of outer chorion and inner vitelline membrane formed by the follicle cells while the eggs are inside the ovariole. The present paper deals with the histological structure of egg of an Orthopteran insect, *Poekilocerus pictus* as revealed under light microscope (LM) as well as under transmission electron microscope (TEM).

MATERIALS AND METHODS:

Live *Poekilocerus pictus* has been collected from wild fields and reared in insect rearing cage. Immature and mature females were dissected in orthopteran saline (Clayton *et al*., 1958) under a stereoscopic binocular microscope and eggs have been obtained. Morphometry has been done with the aid of slide calipers.

[16] Assistant Professor., Zoology, Darjeeling Govt. College, Darjeeling, West Bengal, India.

For light microscopy (LM) first of all the Bouin's fluid-fixed eggs were washed in distilled water and then dehydrated in ascending grade of ethyl alcohol. Then the specimens were cleaned with xylene and after that passed through half-xylene half-paraffin stage and finally twice in full-paraffin stage. Next paraffin blocks were prepared and after trimming ribbons of paraffin containing egg sections have been obtained by the aid of a rotating type of microtome (1090A, WESWOX, DPTIK). Next paraffin sections have been fixed over slides and then after stretching the slides were passed first through xylene and next through absolute and 90% alcohol. Eosin stain already prepared in 90% alcohol was used to stain the slides. After washing in 90% alcohol the slides have been passed through absolute alcohol twice and in next stage they were mounted by means of DPX. Microscopical examination of the slides was made with the aid of MAGNUS (Olympus) microscope of model number MLX 521251 at the laboratory of the P. G. Department of Zoology, Darjeeling Government College. The eyepiece of the microscope was provided with WF 10X lens, and the objective lenses were of four types - a) 4 (0.10), b) Plan 10 Ph/ 0.25**.** 160/0.17, c) Plan 20 Ph/ 0.40**.** 160/0.17, d) Plan 40 Ph/ 0.65**.** 160/0.17. The microscope was attached to a computer device for digital photography. This device took all the photographs of light microscopic sections.

For transmission electron microscopy (TEM) eggs were primarily fixed in 2.5% glutaraldehyde in 0.12 M Millonig's phosphate buffer for four hours at 4^{o}C. Then they were secondarily fixed (post fixation) in 1% aquas osmium tetroxide for 15 minutes at 4^{o}C and dehydrated by ascending grades of alcohols (30%, 50%, 70%, 90% and absolute). Before embedding samples were immersed in transitional fluid (epoxypropane). Embeddings were done in araldite. Rough trimming were made by glass knives and then ultra thin sections (60nm) were obtained from the ultramicrotome (LKB Bromma with Olympus microscope). Ultra thin sections were collected on golden grids and the grids containing the sections were stained by uranyl acetate and lead citrate. The grids (both unstained and stained) were dried in desicator and then viewed under transmission electron microscope (FEI FP 5018/40 TECNAI G2 Spirit Bio Twin) at IICB, Jadavpur.

RESULTS:

In *Poekilocerus pictus* there are two ovaries of unequal size (Dey and Raziuddin, 2008 a) and each ovary is consisting of many ovarioles. Each ovariole contains a linear series of oocytes in different stages of development; however, it is only the basal oocytes which incorporate yolk and gradually develop into eggs. Changes in the length and breadth of the basal oocyte are shown in Table 1. In the following description we intend to describe the histological changes in the basal oocyte.

In freshly moulted specimens the basal oocyte measures 0.856 ± 0.013 mm X 0.198 ± 0.07 mm (mean ± SD). At this stage the epithelium consists of closely packed low-columnar cells (6.0 – 7.0 µ in length) and the ooplasm of the oocytes is finely granular without any trace of yolk (Fig. 2). The shape of the basal egg follicle is elliptical (Figs. 1 and 6). It contains a centrally placed nucleus with loose granular chromatin. On the third day the basal oocyte attains a length of 1.308 ± 0.007 mm (mean ± SD). The follicle cells surrounding the oocytes are by now slightly larger, 8.5 – 9.5 µ in length, and appear low-cuboidal. The ooplasm is still finely granular without yolk granules and it basically resembles that of day one specimens. On day 5 (Fig. 3) histology of the basal egg follicle is similar to that of three-day-old females except that a slight increase in the dimension of follicular cells has been noted. In this species vitellogenesis starts on day 7 when in the ooplasm small yolk globules make their appearance in the peripheral ooplasm (Fig. 4). Thus during previtellogenic phase the basal oocyte attains critical size and is ready for yolk deposition. In these females the basal oocytes measure 2.206 ± 0.008 mm (mean ± SD) in length and 0.294 ± 0.004 mm (mean ± SD) in breadth. As soon as the basal oocyte becomes vitellogenic, the follicular cells increase in dimension and become distinctly columnar; these cells are now 20 – 26 µ in length with distinct large nuclei, 9.5 – 10.5 µ in diameter, occupying a major part of the cell. The nucleus contained dense chromatin.

In the vitellogenic oocytes fissures between the follicular cells make their appearance, and these appear to form channels for the movement of extraovarial proteins from the haemolymph into the ooplasm. This is also evident from the fact that a number of protein yolk spheres appear in the

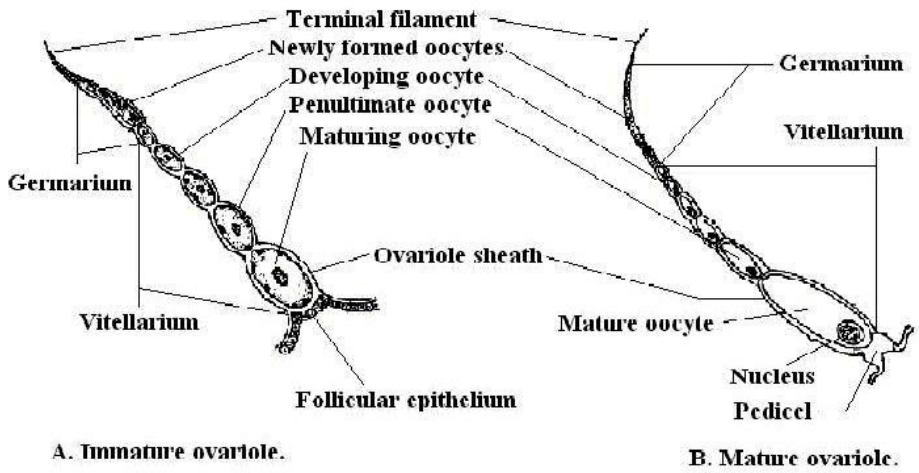

Fig. 1: Diagrammatic representation of the immature (A) and mature (B) ovariole of *Poekilocerus pictus* Fabricius.

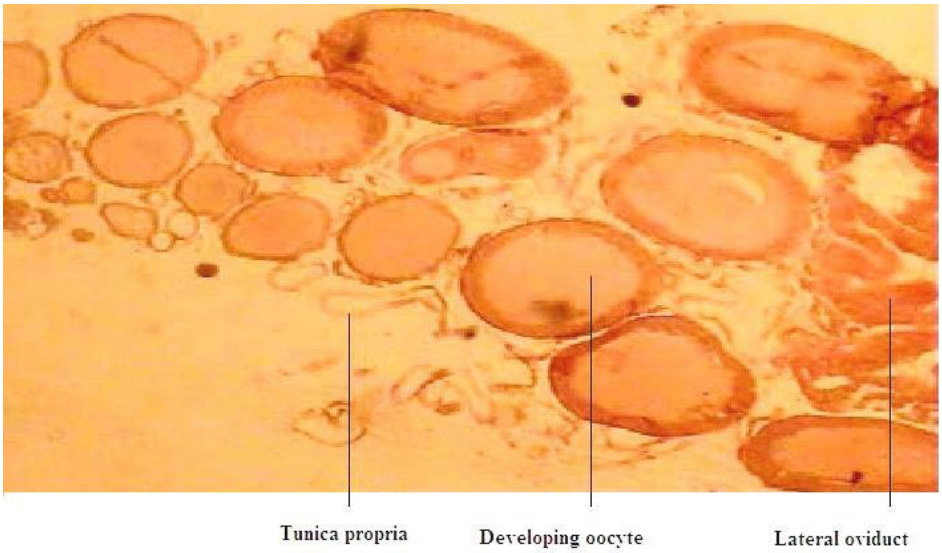

Fig. 2: T. S. of immature ovariole of *P. pictus* (X 450)

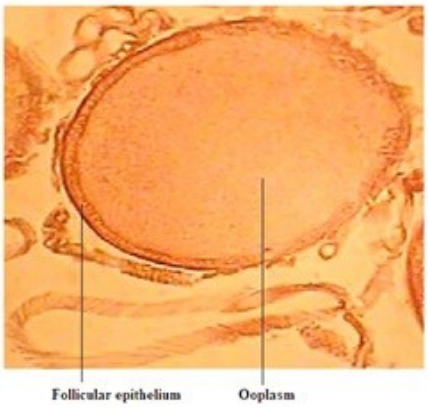

Fig. 3: T. S. 5 day old oocyte of *P. pictus* (X 450)

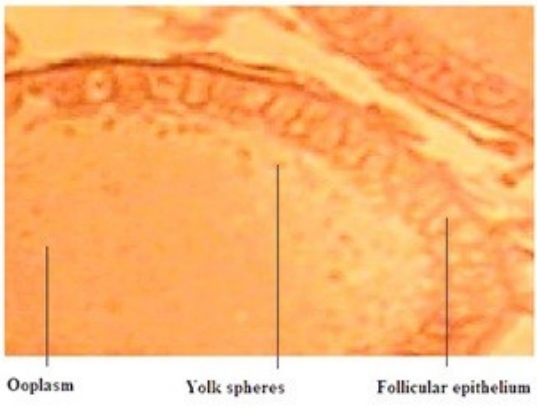

Fig. 4: T. S. of 7 day old oocyte of *P. pictus* (X 450)

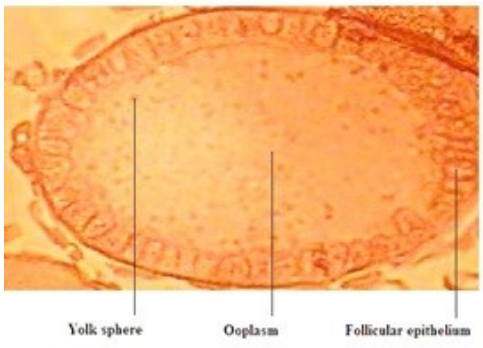

Fig. 5: T. S. of 9 day old oocyte of *P. pictus* (X 450)

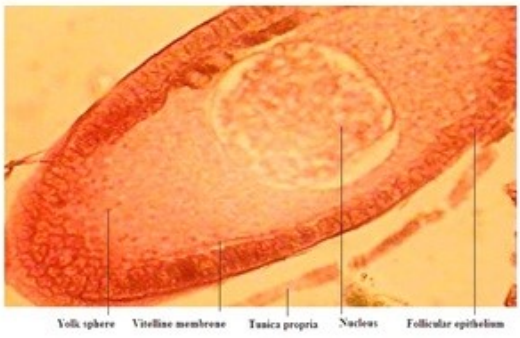

Fig. 6: L. S. of mature egg of *P. pictus* (450)

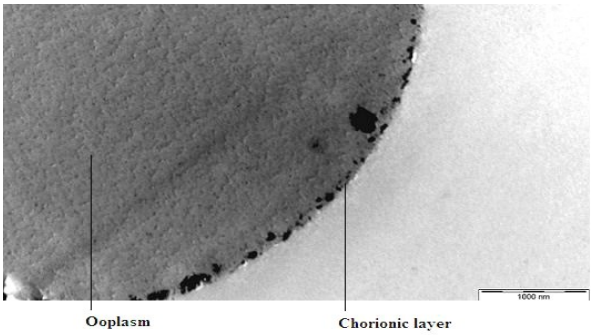

Fig. 7: TEM microphotograph of mature egg of *P. pictus* (Unstained, X 6000)

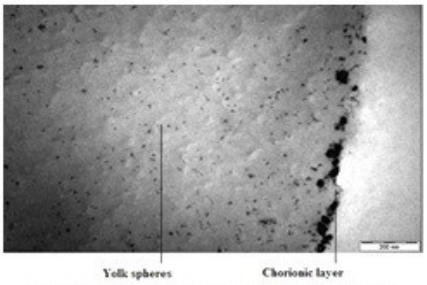

Fig. 8: TEM microphotograph of mature egg of *P. pictus* (Unstained, X 60000)

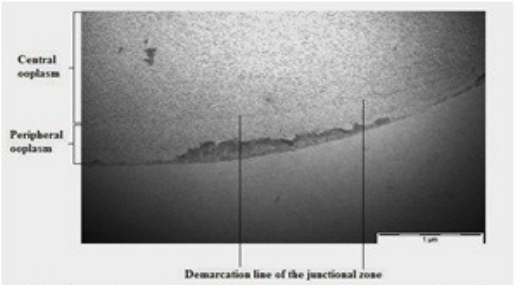

Fig. 9: TEM microphotograph of mature egg of *P. pictus* showing central and peripheral ooplasm demarcated by a junctional zone (Stained, X 18500)

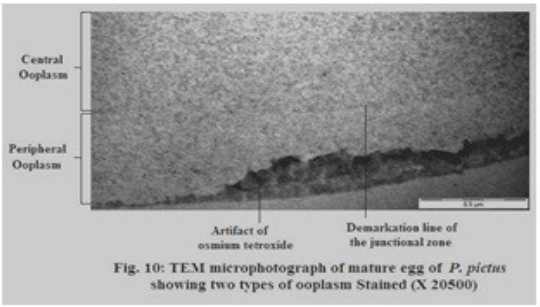

Fig. 10: TEM microphotograph of mature egg of *P. pictus* showing two types of ooplasm Stained (X 20500)

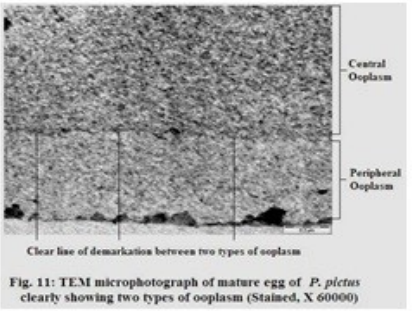

Fig. 11: TEM microphotograph of mature egg of *P. pictus* clearly showing two types of ooplasm (Stained, X 60000)

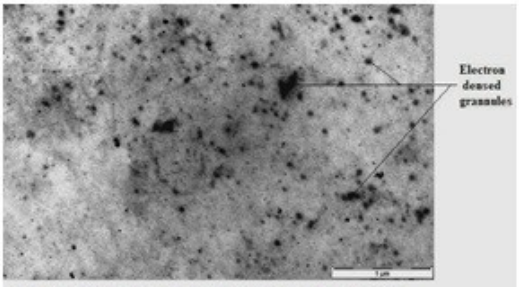

Fig. 12: TEM microphotograph of mature egg of *P. pictus* showing electron densed grannules (Stained, X 26500)

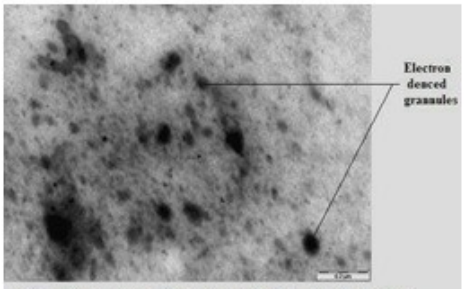

Fig. 13: TEM microphotograph of mature egg of *P. pictus* showing electron denced grannules (Stained, X 60000)

TABLE 1: THE CHANGES IN LENGTH AND BRIDTH OF BASAL OOCYTES WITH AGE IN *POEKILOCERUS PICTUS* FABRICIUS

Age in days	Length (mm)	Mean length	SD	Breadth (mm)	Mean breadth	SD
Just moulted	0.84 0.85 0.84 0.83 0.87	0.856	±0.013	0.21 0.19 0.20 0.19 0.20	0.198	±0.007
3	1.30 1.31 1.30 1.32 1.31	1.308	±0.007	0.21 0.22 0.21 0.23 0.22	0.218	±0.007
5	1.51 1.50 1.49 1.50 1.51	1.502	±0.007	0.25 0.26 0.23 0.24 0.25	0.246	±0.010
7	2.20 2.22 2.21 2.20 2.20	2.206	±0.008	0.29 0.30 0.30 0.29 0.29	0.294	±0.004
9	2.69 2.70 2.69 2.71 2.70	2.698	±0.007	0.30 0.31 0.30 0.32 0.31	0.308	±0.007
11	3.49 3.49 3.51 3.50 3.50	3.498	±0.007	0.58 0.58 0.60 0.59 0.61	0.592	±0.011
13	4.71 4.71 4.70 4.69 4.70	4.702	±0.007	0.72 0.72 0.71 0.70 0.71	0.712	±0.007
15	5.81 5.80 5.81 5.82 5.80	5.808	±0.007	0.91 0.90 0.91 0.92 0.90	0.908	±0.007
17	7.19 7.19 7.20 7.21 7.20	7.198	±0.007	1.19 1.19 1.20 1.21 1.20	1.198	±0.007
19	7.74 7.76 7.75 7.75 7.74	7.748	±0.007	1.39 1.40 1.39 1.39 1.38	1.39	±0.006
21	8.00 8.02 7.99 7.98 7.96	7.99	±0.02	1.52 1.51 1.50 1.49 1.50	1.504	±0.010

ooplasm quite adjacent to these channels. From day 7 onward the basal oocyte is actively engaged in the incorporation of yolk and as such there is an enormous increase in the amount of yolk globules in the ooplasm (Fig. 5). The oocyte rapidly grows in size. The basic histological feature of the oocyte remains the same as described above for the day 7 specimen. However, from day 15 (oocyte size 5.808 ± 0.007 mm X 0.908 ± 0.007 mm) onward the epithelial cells gradually decrease in height and finally in mature follicle (7.99 ± 0.02 mm X 1.504 ± 0.01 mm) these become almost flattened. With the incorporation of yolk into the ooplasm the oocyte nucleus gradually becomes eccentric and finally in the mature egg it lies at the posterior pole of the egg (Fig. 6).

TEM studies of mature egg of *P. pictus* are in line with those of the light microscopic studies. However, interestingly, the ooplasm has been found to be divided into two distinct regions, lighter cortical part and denser medullary part. The cortical part varies in thickness from 0.25 to 0.45µ

(Figs. 7, 8, 9 and 10). At higher magnification also (X 60000) the two regions are quite distinct —

the cortical part containing low electron contrast granules and the medullary part containing electron dense granules (Fig. 11).

In mature oocytes at lower magnification (X 18500 and X 20500) the two parts of the ooplasm appear to be separated by an extremely thin membrane; however, at higher magnifications (X 60000) a distinct membrane separating the cortical and medullary part of ooplasm is not discernable (Fig. 11).

In previtellogenic oocytes the ooplasm appears homogenous. But as the oocytes become vitellogenic, cortical and medullary layers become distinct (Figs. 7 to 11).

Eggs in which vitellogenesis has almost been completed had a thin deeply stained follicular epithelium with granules of high electron density. These granules which were concentrated in the follicular epithelium appeared to migrate into the ooplasm where they were found scattered here and there. These granules in the ooplasm were of various dimensions ranging from 10 to 25 nm in diameter (Figs. 8, 12 and 13). These electron-dense granules, despite appearing to originate in the follicular cells, may be post-fixation artifacts.

DISCUSSION:

In the adult female *Poekilocerus pictus* the number of egg rudiments per ovariole varies from 8 to 11 and does not appear to increase after day 5 of final eclusione. The observations on this aspect confirm the earlier observation made by Karim (1979) in this species.

In *Poekilocerus pictus* the oocytes passed through the previtellogenic growth phase which lasts for six days from the day of fledging during this period the basal oocyte attains the length of just over 2.0 mm. This is the so-called "critical size" of the oocyte which becomes competent for yolk deposition. In this insect vitellogenesis in the basal oocyte commenced on the 7th day after fledging. This phase lasted for 14 to 16 days in the first ovarian cycle. The penultimate oocytes do not become vitellogenic until the basal oocytes are mature and ovulated. These observations are in line with those of Raziuddin and Ghose (1987) and Anwar and Raziuddin (2002) made in *Poekilocerus pictus* and are also in agreement with those of Hill *et al.* (1968) and Tobe and Pratt (1975) on the desert locust *S. gregaria.*

The terminology used by previous workers in connection with the ovariole sheath is both conflicting and confusing. Many workers reported the presence of two ovariole sheaths in insects. The outer sheath has been described by Gross (1903) as a peritoneal sheath and Nelson (1934) referred to an outer thin connective tissue layer as membranea propria. Snodgrass (1935) mentioned an epithelial sheath. Riede (1912) and Wigglesworth (1950) have described a connective tissue sheath and more recently Bonhag (1959) referred to an external ovariole sheath. Just below it lies the second sheath which is a membranous and non-cellular basement membrane covering each ovariole and is termed the tunica propria. In *Poekilocerus pictus* the ovariole has two thin covers, the outer ovariole sheath and the inner basement membrane line immediately beneath the sheath. As usual in *Poekilocerus pictus* the follicular epitheliums are initially formed of small cuboidal cells which, during vitellogenesis, become columnar and, in oocytes which are nearing completion of vitellogenesis, become flattened.

Ovariole surface in previtellogenic stage of *Poekilocerus pictus,* according to SEM studies (Dey and Raziuddin, 2008 b), is highly convoluted longitudinally, but as vitellogenesis starts and proceeds in the basal oocytes, the surface folds distinctly decrease in dimension and pores of variable dimension and shapes, which were totally absent in the previtellogenic stage, appear in patches. These pores are supposed to help in the transport of extraovarial vitellogenins (vitellogenic proteins) into the ooplasm of the growing basal oocytes. Further, in high resolution scanning electron micrographs 9-11 nm wide cracks have been seen which may either be artifacts or passages for vitellogenin entry (Dey and Raziuddin, 2008 b).

TEM studies of mature egg of *Poekilocerus pictus* show that the ooplasm of the egg is distinctly divided into a lighter cortical part containing granules of low electron density, and a denser medullary part containing granules with high electron density. Cytoplasmic qualities in the egg have been studied by a few workers through ultrastructural analysis (see, Buning, 1994; Al-Dawsary *et al*., 2013). In the flea,

Hystrichopsylla talpae Buning and Sohst (1988, 1989) have reported that in early previtellogenic stage there is a homogenous cytoplasm which is very poor in free ribosomes but gradually and slowly a belt of ribosome-rich cytoplasm appears around the oocyte nucleus indicating a low rate of synthesis of rRNA and ribosomes; however, later on ribosome-rich cytoplasm contributes to more than 90 percent of the final euplasm at the end of previtellogenic growth. In mosquitoes the cortical layer of ooplasm beneath the plasma membrane is almost free of ribosomes and mitochondria and consists of filamentous matrix (Clements, 1992). Further, coated vesicles of 120-140 nm diameters are the main constituents of the cortical layer (Raikhel and Lea, 1985). We expect a similar situation as described above to exist in the developing eggs of *Poekilocerus pictus*, in which the denser part of the ooplasm appears to be very rich in free ribosomes.

ACKNOWLEDGEMENTS:

Author is thankful to the Director, Indian Institute of Chemical Biology, Jadavpur, Kolkata, India for providing facilities of Transmission Electron Microscopy and Professor (Dr.) Dipak Ranjan Mandal, Director of Public Instructions, Government of West Bengal for his financial assistance and Professor (Dr.) Lalita Rai Ahmed, Principal, Darjeeling Government College, Darjeeling, West Bengal, India for her keen interest in this study.

REFERENCES:

1. **Al-Dawsary, M. M.; Moursy, E. B. and AlBakairi, A. M. (2013):** Histological Characterization and Embryonic Development In the fertilizing eggs of the Red Palm Weevil, *Rhynchophorus ferrugineus* (Oliver). *Nature and Science*. **11(12)**: 90-98.
2. **Anwar, M.S. And Raziuddin, M. (2002):** Effect of photoperiod on öocyte development in a pyrgomorphid grasshopper, *Poekilocerus pictus* Fabricius. *Indian. J. Environ. And Ecoplan*. **6 (1):** 29-32
3. **Bonhag, P.F. (1959):** Histological and histochemical studies on the ovary of the American cockroach *Periplaneta americana* (L). *Univ. Calif.Publ. Entomol.* **16**: 81-124
4. **Buning, J. (1994):** The insect ovary- ultrastructure, previtellogenic growth and evolution. *Chapman and Hall, London, UK.*
5. **Buning, J. and Sohst, S. (1988):** The flea ovary: Ultrastructure and analysis of cell clusters. *Tissue Cell,* **20**: 783-795
6. **Buning, J. and Sohst, S. (1989):** The ovary of *Hystrichopsylla*: Structure and previtellogenic growth, in *Regulation of Insect Reproduction IV 1987,* (eds M. Tonner, T. Soldan, B. Bennettova), *Academia Praha,* pp 113-124
7. **Chapman, R.F. (2000):** The Insects, Structure and Function (4th Edition). *Cambridge University Press*
8. **Clayton, B.P., Deutsch, K. and Jordan – Luke, B.M. (1958):** The spermatid of the House Cricket *Acheta domisticus. Quart. J. Micr. Sci*. **99**: 15- 23
9. **Clements, A. N. (1992):** The Biology of Mosquitoes Vol.I, Development, Nutrition and Reproduction. *Chapman & Hall, London.*
10. **Dey, S. and Raziuddin, M. (2008 a):** Oocyte resorption in the pyrgomorphid grasshopper, *Poekilocerus pictus* Fabricius. *Proc. Zool. Soc. India* **7(2)**: 27-39

11. **Dey, S. and Raziuddin, M. (2008 b):** Surface architecture of egg of a pyrgomorphid grasshopper, *Poekilocerus pictus* Fabricius. *The Bioscan* 3 (3) : 357 – 360
12. **Gross, J. (1903):** Unter su chungen uber die Histologie des Insectenovariums. *Zool.JI.Anat*.**18**: 71-186
13. **Hill, L., Luntz, A.J. and Steele, P.A. (1968):** The relationship between somatic growth, Ovarian growth and feeding activity in the desert locust. *J.Insect Physiol.* **14**, 1-20
14. **Jackson, J. T., Tarpy, D. R. and Fahrbach, S. E. (2010):** Histological estimates of ovariole number in honey bee queens, *Apis mellifera,* reveal lack of correlation with other queen quality measures. *Journal of Insect Science*: Vol. **11**, Article **82**: 1 - 11
15. **Karim, S.W. (1979):** Studies on the structural and histochemical changes in the reproductive organs during postembryonic development of *Poekilocerus pictis* (Fabr.). *Ph D Thesis. Magadh University, Bodh-Gaya.*
16. **Khurana, A.D. (1975):** Alternative host plants of Aak grasshopper, *Poekilocerus pictus* (Fabricius). *Entomologists Newsletter.* **5** (6-7):34
17. **Nelsen, O.E. (1934):** The development of the ovary in the grasshopper, *Melanoplus differentialis* (Acrididae: Orthoptera). *J. Morph.* **55:** 515-543
18. **Parihar, D.R. (1974):** Some observations on the life history of Aak grasshopper, *Poekilocerus pictus* (Acridoidea: Pyrgomorphidae) at Jaipur, Rajasthan, India. *J. Zool. Soc. India.* **26**: 89-129
19. **Pruthi, H.S. and Nigam, L.N. (1939):** The bionomics, life history and control of the grasshopper, *Poekilocerus pictus* (Fab.) a new pest of cultivated crops in north India. *Indian J. Agric. Sci.***9**: 629-641
20. **Pruthi, H.S. (1954):** The Aak grasshopper- *Poekilocerus pictus* (Fabr.) (Acrididae) attacking Citrus in Delhi. *Indian J. Ent.* **16**: 196
21. **Raikhel, A.S. and Lea, A.O. (1985):** Hormone-mediated formation of the endocytic complex in mosquito oocytes. *Gen. Comp. Endocrinol,* **57**: 422-33
22. **Raziuddin, M. and Ghose, I.K. (1987):** Stomodial nervous system of a pyrgomorphid grasshopper, *Poekilocerus pictus* Fabricius. *The Indian Zoologist, Vol.***11,** Nos. 1&2, pp. 105-109
23. **Raziuddin, M., Singh, S.B.; Sharma, A.K. and Singh, B.K. (1991):** Feeding habits of *Poekilocerus pictus* Fabricius (Acridoidea; Pyrgomorphidae). *Environment and Ecology.* **9** (1): 100-102
24. **Raziuddin, M. and Anwar, M.S. (1997):** On the occurrence of a pyrgomorphid grasshopper, *Poekilocerus pictus* Fabricius in India with particular reference to Bihar. *Columban J. Life Sci.* **5** (1-2): 205-211
25. **Riede, E. (1912):** Vergleichende unter suchung der saunerstoff Versorgung in den Insektenovarien. *Zool. Jahrb. Abt. Zool.* **32**: 231-310
26. **Snodgrass, R.E. (1935):** The abdominal mechanism of grasshoppers. *Smithson.Misc. coll.* **94**: 1-89
27. **Tembhare, D.B. (2006):** Modern entomology. *Himalaya Publishing House, Mumbai.*
28. **Tobe, S.S. and Pratt, G.E. (1975):** Corpus allatum activity *in vitro* during ovarian maturation in the desert locust, *Schistocerca gregaria. J. exp. Biol.* **62**: 611-627
29. **Wigglesworth, V.B. (1950):** *The principles of insect physiology.* London. pp 554
30. **Wigglesworth, V.B. (1965):** *The principles of insect physiology.* Methuen, London