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Histological and histochemical changes in oocytes during oogenesis in *Orthetrum sabina* (Drury 1770) (Anisoptera: Libellulidae)

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Abstract

This study presents the oocyte development of *Orthetrum sabina* with the dynamics of histology and histochemistry. The ovary is panoistic type which contain many ovariole. Ovariole contain terminal filament, germarium, vitellarium. Five stages of oocytes were identified based on nature of cytoplasm, presence of germ vesicle, yolk granules and chorion. Spherical oval shaped oocyte I gradually increases in size and attained elliptical shaped oocyte V. Germinal vesicle is conspicuous in oocyte I and II but shifted towards one pole in oocyte III and completely disappear in oocyte IV. Chorion around plasma membrane manifested in oocyte IV. Proteins and carbohydrates entered in ooplasm from early stage while lipid appear late during oocyte development. Proteins, carbohydrates and lipid were demonstrated in oocytes by mercury bromophenol blue method, periodic acid Schiff method and Sudan black B method respectively.

Keywords: Vitellogenesis, oocyte development, ovariole

Introduction

In insect, ovaries containing ovarioles distinguish into two categories i. e., panoistic without nutritive cell and meroistic with nutritive cell. In Lepidoptera, and Diptera meroistic ovarioles are found^[1]. In apterygotes, grasshoppers, dragonflies, termites, stoneflies, crickets, fleas and some beetles panoistic type ovarioles are present^[2, 3, 4]. Vitellogenesis is the amount of rapid gametocyte growth period in which the deposition of yolk occurs. The yolk is a complex substance composed of proteins, lipids, carbohydrates and other minor components found in the oocyte^[5]. Oocyte growth is enhanced by the endocytosis of yolk proteins that can be derived from two sources, the fat body vitellogenins and the complicated follicular epithelial cells surrounding the oocyte-nurse cell^[6, 7, 8]. The oocyte surface is not exposed directly to the hemolymph, but it is covered by follicular epithelium. Large channels form between the epithelial cells, a condition called patency that allows the passage of hemolymph-derived proteins to the oocyte surface and into the oocyte through the follicular epithelium^[9, 10, 11, 12]. During previtellogenic stage macromolecules and organelles are supplied to oocytes by trophocytes^[13]. The oogenesis process is fundamental to understanding the number of egg cycles, egg-laying patterns, and potential fecundity of panoistic containing during different seasons. In insects, glycogen and follicle cells, glycoaminoglycons are synthesized during vitellogenesis by the ovary. These two compounds react with vitellogenin hemolymph to form a complex that is absorbed by oocytes^[14, 15]. Thus the protein and lipid yolks were exogenous whereas the carbohydrate yolk is endogenous in insects as observed by^[16].

Materials and Methods

Insect collection

The dragonfly species, *Orthetrum sabina* (Drury 1770) (Anisoptera: Libellulidae) were gathered through traditional net sweeping, method during their hovering activities. During the month of July-January, from 2016-2018, a survey was carried out at the various water reservoirs in central India (21 ° 08'59.4"N 79 ° 04'50.1"E). For further studies, the samples collected were preserved in 70 % alcohol. The experimental protocol accepted prior to the start of the research by RTM Nagpur University's Institutional Animal Ethics Committee, Nagpur (Registration no.- 478/01/a/CPCSEA).

Morphological preparation

Collected species were executed in the laboratory and freshly dissected in Ringer's solution. Dissections were performed under a binocular stereoscopic microscope (Karl Zeiss). For morphological preparations, the ovary has been washed multiple times with distilled water, photographs taken at a magnification of 4 x.

Histological preparation

After dissections of *O. sabina* ovaries were fixed in Bouin's fixative for 24 hours. The tissue was dehydrated with graded series of alcohol (70 %, 90 %, 100 %), ovaries transfer into xylene for transparency and embedded in parafine wax. Tissue was cut in microtome sectioned at 5 µm thickness and stained by Harris Haematoxylin Eosin (HE) to perceive the general histological view.

Histochemical preparation

Periodic Acid Schiff (PAS) and Mercury Bromophenol Blue (MBB) Sudan Black B (SSB) were used to mark carbohydrate, protein and lipid in the oocytes, respectively as previously suggested [17]. Sections were examined microscopically in the microscope (Karl Zeiss) and photographed in Tuscon camera.

Results

The ovary consists of 200 to 250 number of ovarioles, which consist of three main region terminal filament, germarium and vitellarium (Fig. 1A). Each ovariole is lined with a follicular epithelium and covered by two membranes: the tunica propria and the external ovariole sheath (Fig. 1A 1B). The antero-posteriorly linear development of the egg can be clearly demarcated in the ovarioles (Fig. 1A). The entire process of vitellogenesis is divided into five developmental stages- pre-vitellogenic, early vitellogenic mid-vitellogenic, late vitellogenic and maturation.

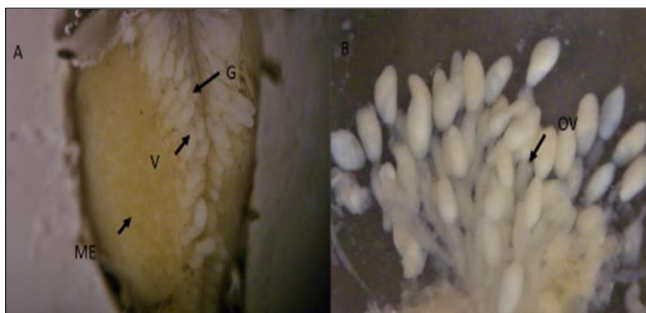


Fig 1: Morphological structure of ovary of *O. sabina*. A) ovary with germarium (G) vitellarium (V) and mature eggs (ME). B) Ovarioles (OV)

Oocyte I

These oocytes were small in size and morphologically differ from round to rectangular. Some time it shows irregular shape. Homogeneous cytoplasm and germ vesicles representing the nucleus, and the plasma membrane is thin (Fig. 2A).

Oocyte II

The size of oocytes increases as compared to oocyte I and morphologically more or less rounded in shape. The plasma membrane is showing more thickness where follicular epithelium cell has encircled the oocyte. There is a fine granulation observed which dispersed throughout the

cytoplasm and the presence of germ vesicle and nucleoli is demarcated (Fig. 2B).

Oocyte III

The size of oocytes increases more rapidly as compared to oocyte I and oocyte II. Morphology of oocytes also changes with rounded, elliptical and irregular shapes. The cytoplasm hold within an unrefined granulation and germ vesicle comes into sight located at the pole. The vitelline membrane is rapidly produced on the surface of the oocyte by the active synthesis and subsequent secretion of granular osmiophilic material from the enveloping follicle cells after the maximum oocyte size has been reached (Fig. 2C).

Oocyte IV

The size of oocyte gets increases. The cytoplasm is completely filled with yolk granule of the different size where larger size granules appear in the periphery and the central region is filled with smaller granules. Germ vesicle is completely diminished in oocyte IV. The formation of the vitelline membrane is continued by follicular epithelial cell and chorion appeared in oocyte IV (Fig. 2D).

Oocyte V

Oocyte reached a maximum developmental stage with maximum size. The cytoplasm packed with different yolk granules throughout the oocytes. Germ vesicles is completely disappeared. Follicle epithelial cell decreases in size (Fig. 2E).

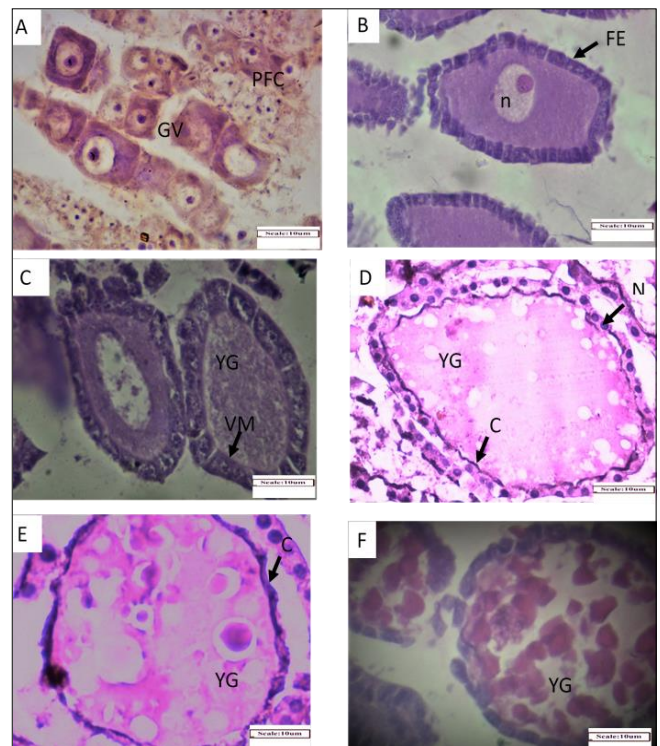


Fig 2: (A to F) Histochemical section of *Orthetrum sabina* ovary stained by hematoxylin, Eosin. Detail of oocyte I, II, III, IV, V. Pre follicular cells (PFC), Germ vesicle (GV), Nucleolus (n) Nucleus (N) yolk granules (YG), follicular epithelium (FE), Chorion (C), Vitelline membrane (VM). Scale bar: 10µm.

2. Histochemical demonstration of the ovary

2a. Proteins

Oocyte I in the germarium reacted positively to MBB. The cytoplasm shows a dark stain as compared to the germinal

vesicle where the nucleolus shows a highly positive reaction with MBB (Fig. 3A).

Oocyte II stage shows the positive stain towards the cytoplasm. Germinal vesicle show feable reaction than the cytoplasm. The nucleolus is showing a strong positive reaction (Fig. 3B).

Oocyte stage III shows strongly stained yolk granulation. The follicular epithelial layer cells show a positive reaction. Cytoplasm of follicular epithelium cell show light stain and the nucleus show a highly positive stain for MBB. The vitelline membrane shows a positive reaction for MBB (Fig.

3C).

Oocyte stage IV shows the strong positive reaction of yolk granule with MBB. In follicular epithelium cell cytoplasm show, the rare stain of MBB but the nucleus is intensely stained with MBB. The vitelline membrane shows a strong positive reaction for MBB (Fig. 3D).

Oocyte Stage V shows strongly stained yolk granulation which indicates high protein content (Fig. 3E). The chorion and vitelline membrane show a strong positive reaction which shows its proteic nature (Fig. 3F).

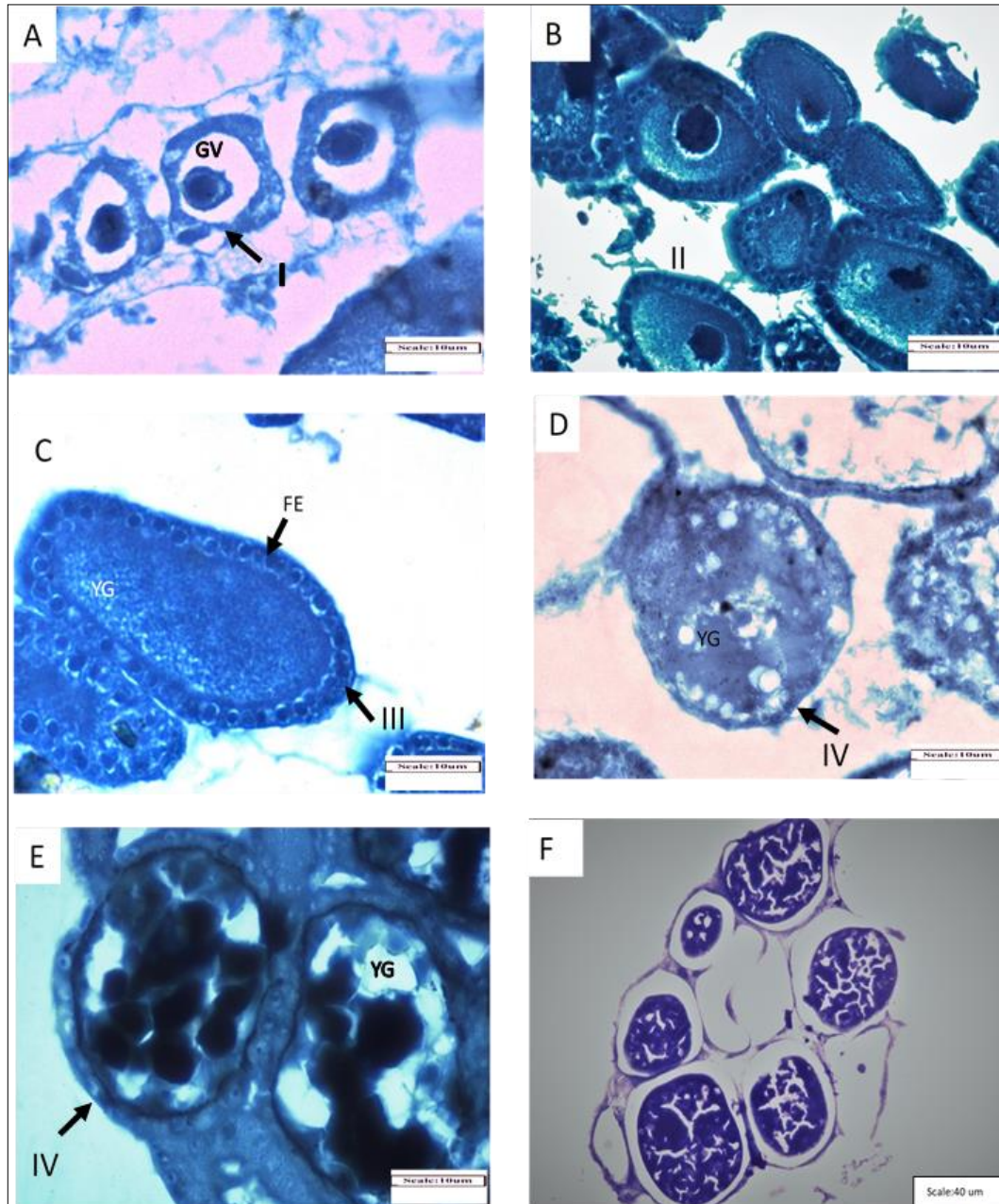


Fig 3: (A to F) Histochemical section of *Orthetrum sabina* ovary stained by Mercury Bromophenol blue. Detail of oocyte I, II, III, IV, V. Germ vesicle (GV), Nucleus (N) yolk granules (YG), follicular epithelium (FE), chorion (C). Scale bar: 10µm.

2b. Carbohydrate

Oocyte I and oocyte II show positive reaction for cytoplasm and nucleolus for PAS but cytoplasm show weaker reaction than nucleolus for PAS (Fig. 4A - 4B)

Oocyte III shows a weakly positive reaction for PAS. A weakly stain reaction is also seen in follicular epithelium cells where the vitelline membrane also shows a much weaker

reaction than the cytoplasm. The nucleolus show more reaction than the cytoplasm and vitelline membrane (Fig. 4C-4D).

Oocyte IV and oocyte V show strongly stained yolk granules. These granules are having different shapes and sizes. The chorion structure also shows a strong positive reaction for PAS (Fig. 4E-4F).

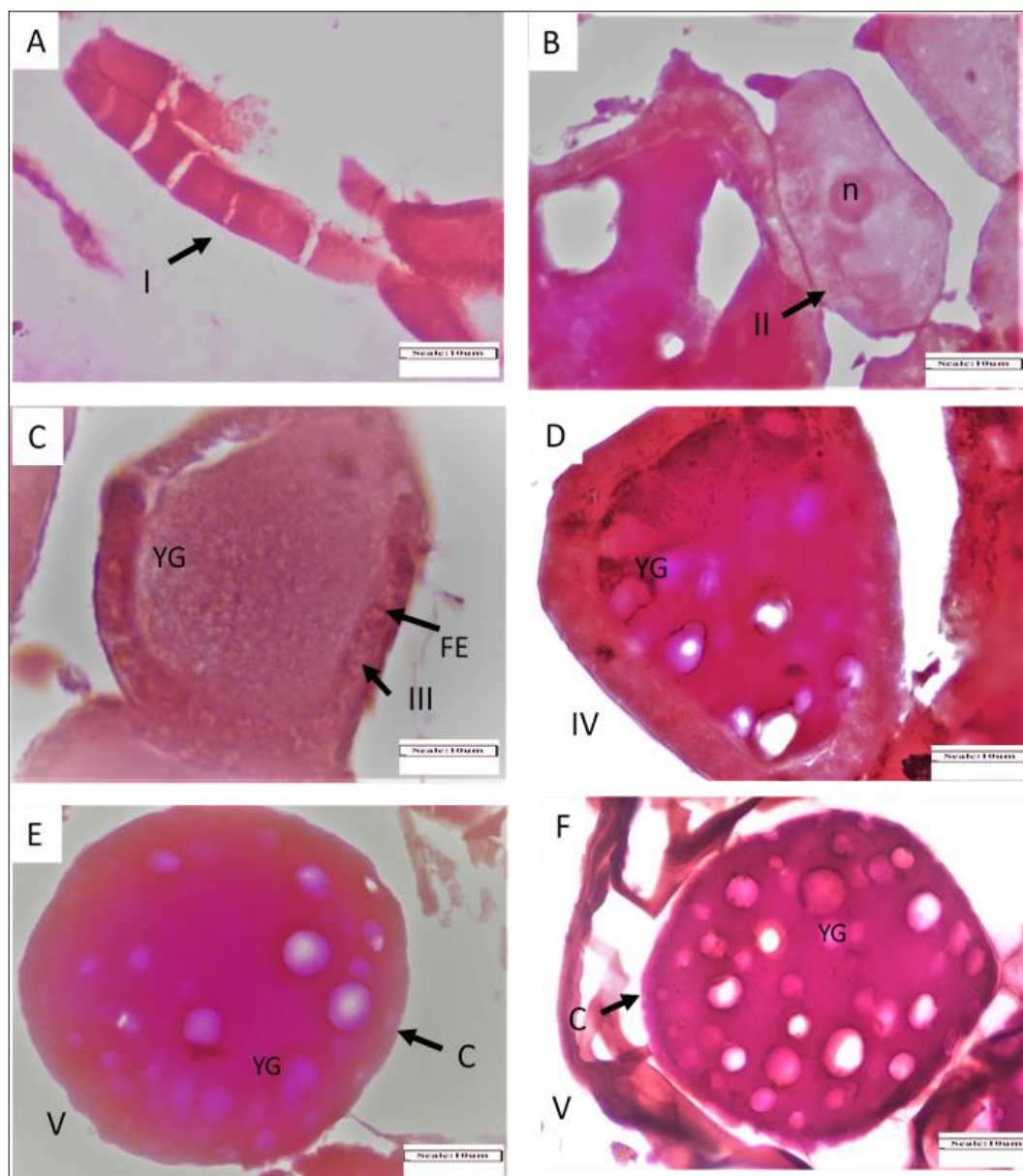


Fig 4: (A to F) Histochemical section of *Orthetrum sabina* ovary stained by Periodic acid schiff. Detail of oocyte I, II, III, IV, V. Germ vesicle (GV), Nucleolus (n) Nucleus (N) yolk granules (YG), follicular epithelium (FE), chorion (C). Scale bar: 10µm.

2c. Lipid

Oocytes stage I show positive reaction of SBB with cytoplasm, germinal vesicle, nucleolus and plasma membrane (Fig. 5A).

Oocytes II stage also shows a positive reaction with the SBB. Fine yolk granulation scattered throughout the cytoplasm was observed. Germ vesicle was also observed with some rare staining (Fig. 5B)

Oocytes III stage show few and small yolk granules scattered in the cytoplasm. The yolk granules do not show a strong reaction with the SBB. The vitelline membrane also shows positive reaction to SBB (Fig. 5C).

Oocytes in stage IV contained a large number of yolk granules that reacted negatively to the SBB (Fig.5D)

Oocytes in stage V shows positive reaction for SBB in chorion (Fig. 5E).

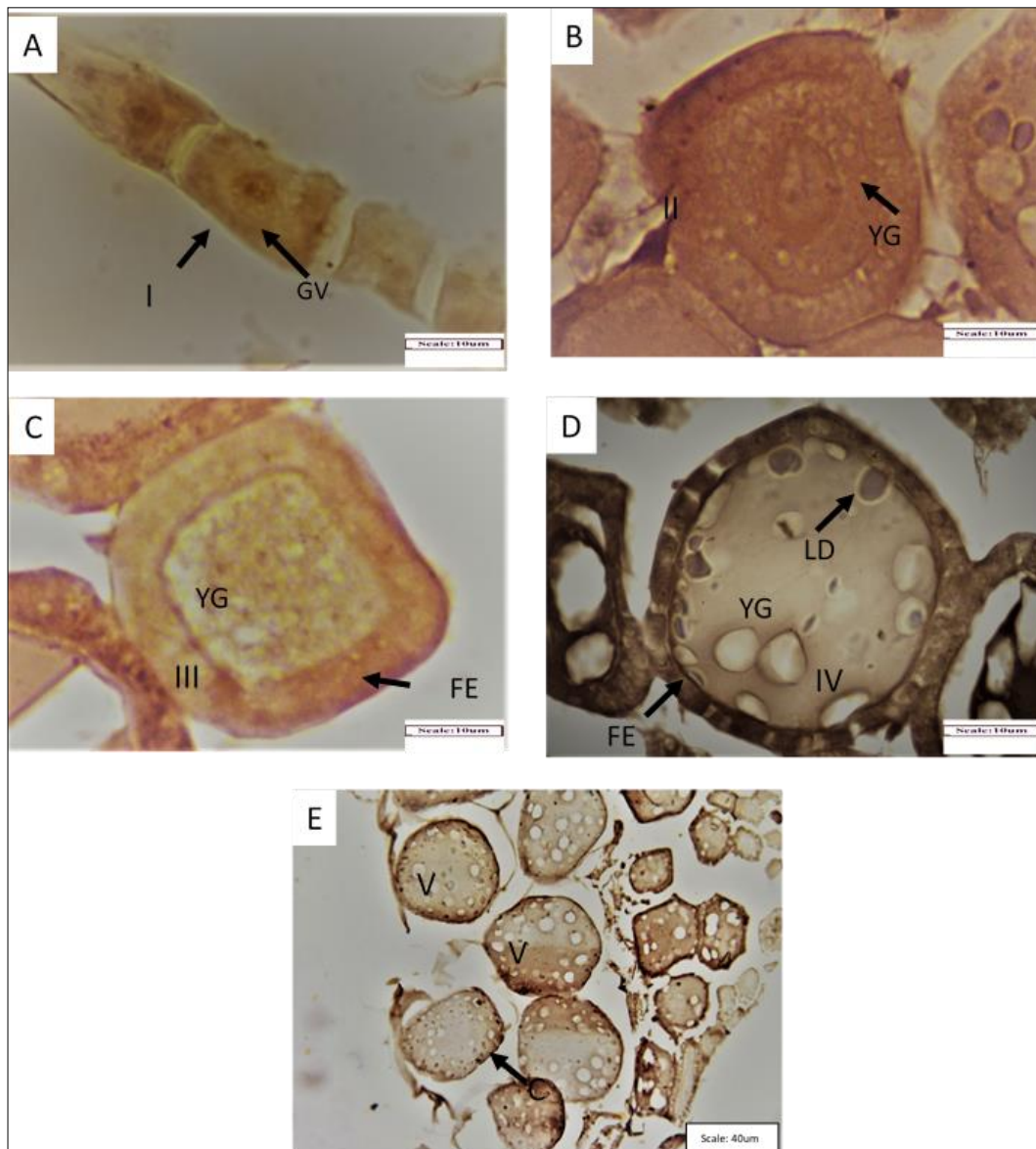


Fig 5: (A to E) Histochemical section of *Orthetrum sabina* ovary stained by Sudan Black B. Detail of oocyte I, II, III, IV, V. Germ vesicle (GV), yolk granules (YG), follicular epithelium (FE), chorion (C) Lipid Droplets (LD). Scale bar: 10µm.

Discussion

In Odonata, ovaries comprises of numerous thread like ovarioles which generally stretched from the first to seventh abdominal segment. In *Pantala flavescens*, the ovarioles run from first abdominal segment to fifth abdominal segment [18]. In *Orthetrum chrysis*, ovarioles are placed in the first to sixth abdominal segment [16] while in *Tramea Virginia*, ovarioles are stretched from second to sixth abdominal segments [19]. In *O. sabina*, ovary is lying from the first to fifth abdominal segment to lateral edge of gut [20]. The ovarioles in Odonata are without nurse cells and thus are of panoistic form. These ovarioles are arranged in an oblique manner. Panoistic ovarioles are either synchronous as in *Amblyomma tirste* showing no marked morphological difference in oocytes [21] or asynchronous as in *Melanogryllus desertus* [22] which show marked morphological variations in oocytes. The process of vitellogenesis in *O. sabina* is divided into five stages i. e., pre-vitellogenesis, early vitellogenesis, mid- vitellogenesis, late vitellogenesis and maturation. The insect ovariole number changes from one species to other i. e., one in Coleopteran and thousand in termites whereas in *O. sabina* the numbers of ovarioles are 200 to 250. At the beginning of vitellogenesis, basal oocyte increases in size and become spherical at the

beginning of late vitellogenesis and maturation. Ovarioles are discriminate into three zones the terminal filament, germarium, and vitellarium. Four regions were reported in the ovarioles of *O. chrysis* [16] and *Tramea virginia* [19]. All along the epithelial lining (vitellogenic stage) the appearance of clear spaces between the apical regions of the follicular cells and ooplasm were observed in *Melanoplus* [23]. At post vitellogenic phase, the follicular cells become secretory and the oocyte develops an inner vitelline membrane and an outer chorion [24]. As in case of *Acheta domesticus* [25], follicular cells become prominent in oocyte stage II in *O. sabina*, and they changes from columnar to cuboidal to squamosal from oocytes II to oocytes V.

Proteins, lipids, and carbohydrates along with other component deposited in the oocytes during oogenesis in stepwise manner [26]. Yolk deposition leads to rapid growth of the oocytes. In insect ooplasm, proteins and carbohydrates combine with each other and form yolk sphere or yolk globules [27]. Proteins begin to enter in ooplasm from oocyte I stage. Appearance of MBB positive material from periphery as well as around the germinal vesicle indicate two source of protein, either exogenous from fat body via follicular cell or endogenous synthesized by germinal vesicle in ooplasm.

Carbohydrates appear first around the germinal vesicle in oocyte I and gradually spread across the cytoplasm as it pass through oocyte II stage to oocyte V stage. Intermixing of proteins and carbohydrates seems to form yolk globules. Thus yolk deposition begins very early from oocyte I stage. On the onset of yolk deposition, oocyte nucleolus degenerated as observed in *D. melanogaster* [28] but such degeneration of nucleolus lacking in *O. sabina* like in *buffalo-fly*, *Lyperosia exigua* [29]. Although contribution of follicular cells in yolk spheres reported in meriostic ovariole of *Glossina austeni* [30], clear role of follicular cells in yolk deposition are not yet been demonstrated in panoistic ovariole. Prominent lipid droplets were appears first in oocyte IV towards the periphery in vicinity of yolk globules as in *Lygus lineolaris* [31] and *Gryllus bimaculatus* [32].

In conclusion, ovariole of *O. sabina* is panoistic as in other dragonflies. Oogenesis is completed through oocyte I, oocyte II, oocyte III, oocyte IV and oocyte V stage and are asynchronous as at a time we observed different stages of oocytes in ovariole of *O. sabina*. Proteins and carbohydrates enter from early stage of oogenesis and form the yolk globules but lipid droplets enter late during oogenesis.

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