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Oogenesis in Eudichogaster Kinneari (Oligochaeta-Annelida): Histological and Histochemical Profile

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Abstract

Four succeeding stages in the Oogenesis were distinguished in Eudichogaster kinneari, based on shape, size and organisation of oocytes. These are undifferentiated, differentiated, previtellogenic and vitellogenic oocytes, as revealed by several histological and histochemical techniques.

Keywords: Eudichogaster kinneari, reproduction, hermaphrodite, gonads, Oogenesis, histomorphology, Histochemistry.

Introduction

Among the annelids sexes are generally separate in polychaetes but oligochaetes are fundamentally hermaphrodite though slightly protogynous. The oligochaetes have evolved a complex reproductive morphology and a repertoire of accessory glandular structures in keeping with their hermaphrodite nature and diverse habits[1].

As oligochaetes are hermaphrodite with separate ovaries and testes, they exhibit an annual reproductive cycle with the gonads maturing in early rainy season, regressing later in the year and maturing again in following early rainy season. A cycle of neurosecretory cell development and activity to begin with post embryonic development has been shown by Herlant- Meewis[2]

Numerous tropical invertebrates exhibit synchrony in breeding times[3]. The reproductive cycle of invertebrates are annually determined on the basis of gonad indices, spawning potential, appearance of mature gametes in gonads, oocyte diameter frequency, the broading of eggs etc[4, 5]. Reproduction in annelids has been studied by few workers[6-13].

Since virtually nothing is known about the oogenesis of the tropical earthworm, Eudichogaster kinneari, therefore the present work was undertaken with a view to know the histology and histochemistry of oogenetic stages in same worms.

Material and Methods

The Earthworms Eudichogaster kinneari of approximately same weight (6.5 + 0.001gm), length (80 to 120mm) and diameter (5-7 mm), were collected from the vicinity of Ujjain city, India. Only healthy and sexually mature specimens (having well developed clitellum) were used for the present study. The worms were maintained in the laboratory in culture pots with moistened soil, decaying leaves and compost manure etc. The observations documented here, were made during the rainy season (June to August), a period including the time of maximum development of gonads. The temperature and pH of soil were noted which was ranged between 22-25^{0}C temperature and pH was 7. Before making the histological preparation the worms were narcotized and female gonads were immersed in saline solution (0.75 % NaCl) for a few minutes to avoid contractions. For general histological and histochemical studies, the female gonads were fixed in Bouin's fluid and 10% formalin. The fixed ovaries were processed for dehydration and blocks were prepared in paraffin wax and sections were cut at 4-5µ. For histological studies Delafield's Haematoxylin-Eosin and Mallory's triple stain were used. For histochemical observations the following techniques were used viz. Periodic Acid Schiff's (PAS) method for the detection of carbohydrates, Alcian Blue (AB) method for the detection of acid muco polysaccharides, Mercuric Bromophenol Blue (Hg-BPP) method for the detection of proteins, Sudan Black B (SBB) technique to trace out the lipids, Luxol Fast (LF) method for the detection of phospholipids and Best Carmine (BC) technique for detection of glycogen have been used.

Results and Discussion

Histomorphological Results: There are two ovaries, one on each side of the ventral nerve cord in the 13th segment. These are creamish or whitish in colour. Each ovary is attached at its basal end to the septum, while the free end floats in the coelom. Each ovary measured about 880 µm in length and 250 µm in width. The basal part of the ovary contains undifferentiated rounded cells, which are followed by a zone of dividing cells while their distal lobulated processes are longer and have ova of various developing stages which are present in linear arrangement, like undifferentiated, than differentiated, previtellogenic and the vitellogenic oocytes. Their arrangement starts from ovarian cord towards periphery. Figure-1.

During Oogenesis changes are noticed in shape, size and organisation of oocytes. Four different succeeding stages have

been noticed in oogenesis.

Stage I. Undifferentiated Oocytes: These are the youngest oocytes, either round or oval in shape, measuring 0-15 µm in size with homogenous ooplasm. These are present in the ovarian cord and are not differentiable, prospective ova not noticeable different from the remaining cells when stained with heamatoxylin-eosin. Figure-1 and 3.

Stage II. Differentiated oocytes: These are bigger in size than the stage-I oocytes. These are spherical to oval in shape. Each oocyte has a single large nucleus and homogenous ooplasm. The diameters of these oocytes measures 16 µm.-31 µm. staining characteristics are similar to those of other cells. Figure-1 and 2.

Stage III. Previtellogenic oocytes: These are much larger than stage I and stage II oocytes of germinal cord., measures from 32 µm to 64 µm or more larger. These are spheroid or round in shape, having a single nucleus with nucleolus and homogeneous ooplasm; there is no sign of yolk accumulation, stain blue with haematoxylin-eosin. The nucleoplasm with some granular patches of nuclear origin is distinguished. Figure-1, 2, 3 and 4.

Stage IV. Vitellogenic oocytes: These are the largest oocytes, measuring 65 µm – 93 µm or larger, which are mostly spherical in shape. These oocytes show transitional stages from the beginning of yolk accumulation to the mature ovum. Yolk stains brilliantly stains with heamatoxylin having very clear nucleus (Figure -1 and 4). Sometimes two or more nuclei are seen in late vitellogenic stages (Figure- 5). The mature oocytes are fully accumulated with yolk (figure-6). Fully grown mature oocytes are surrounded by a thin follicular membrane and are associated with a number of accessory cells derived from the protogonia of the presumption ovary. Figure.-7.

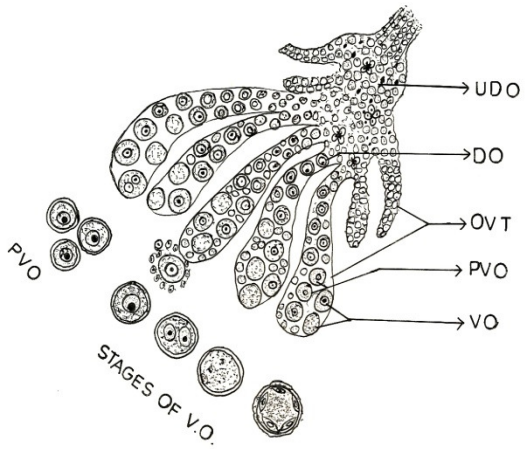
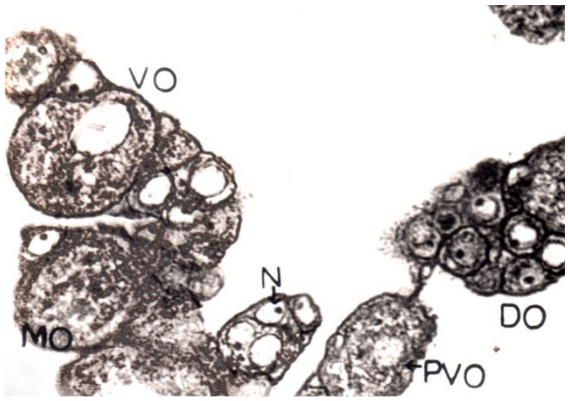


Figure-1
Photomicrograph of T.S. of female gonad of Eudichogaster kinneari showing different stages of oocytes.
OVT: Ovarian Tubule, UDO: Undifferentiated oocytes, DO: Differentiated oocytes, PVO: Previtellogenic oocytes, VO: vitellogenic oocytes.



Figure–2
Photomicrograph of T.S. of female gonad of E.kinneari showing different stages of oocytes
DO: Differentiated oocytes, PVO: Pre vitellogenic oocytes, VO: Vitellogenic oocytes, MO: Mature oocytes, N: Nucleus.

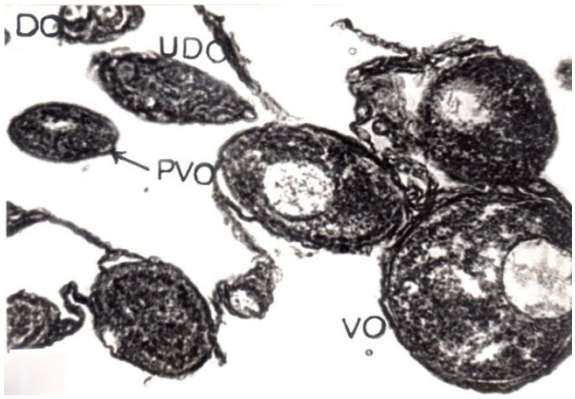
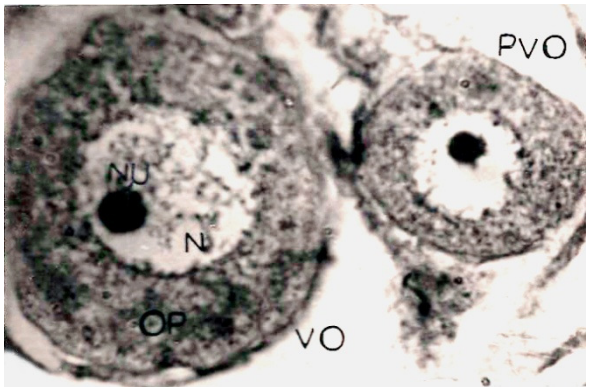
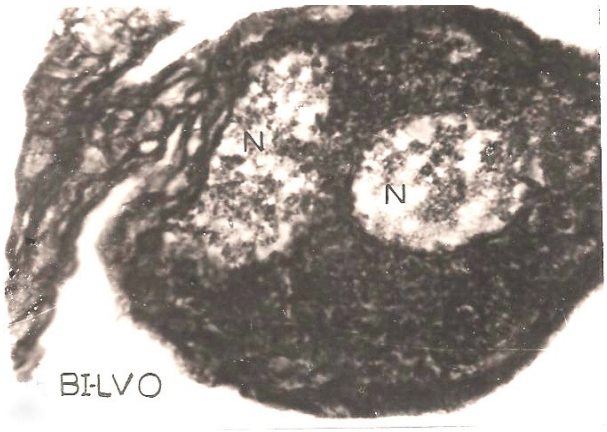


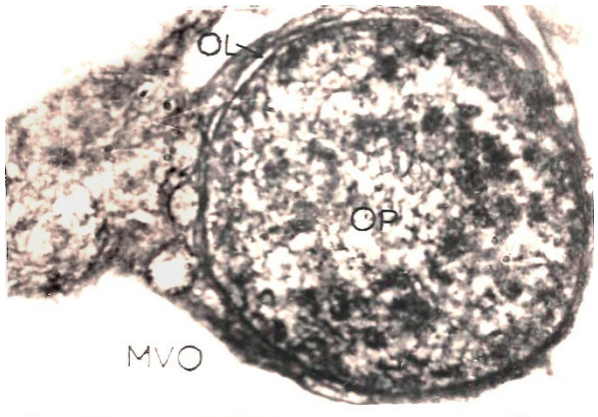
Figure-3
Photomicrograph of T.S. of female gonad showing different oocytes of E.kinneari.
UDO: Undifferentiated Oocytes, DO: Differentiated Oocytes, PVO: Pre vitellogenic Oocytes, VO: Vitellogenic Oocytes.



Figure–4
Photomicrograph of T.S. of female gonad of E.kinneari showing oocytes.
PVO: Previtellogenic oocytes, VO: Vitellogenic oocytes, N: Nucleus, NU: Nucleolus.



Figure–5
Photomicrograph of T.S. Of female gonad of E.kinneari showing Binucleated,
Late Vitellogenic Oocyte, BI-LVO: Binucleated-Late Vitellogenic oocyte, N: Nucleus.



Figure–6
Photomicrograph showing oocyte of E.kinneari
MVO: Mature Vitellogenic Oocyte, OP: Ooplasm, OL: Oolema.

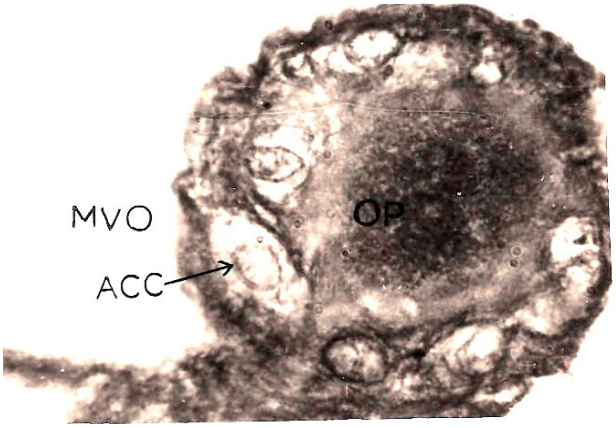


Figure-7
T.S. Female gonad of E.kinneari showing fully grown mature Oocyte with accessory cells

Histochemical Results: With periodic acid Schiff's technique nucleus, nucleolus and ooplasm of all stages of oocytes showed mild positive reactions which suggest the presence of least quantity of carbohydrates and with Alcian blue showed negative reactions, which signifies absence of acid mucopolysaccharides. Mercuric Bromophenol blue test (Hg-BPB) revealed moderate positive results, indicating the presence of sufficient quantity of proteins. Lipids and phospholipids also have been traced in sufficient quantities evidenced by Luxol fast (LF) and with Best Carmine (BC) techniques and Presence of glycogen was also observed in less quantity with Sudan Black B (SBB) technique. Table 1.

Table-1
Histochemistry of Different Stages of Oocytes in Eudichogaster kinneari

Histochemical tests	Oocytes			
	Stage-I	Stage-II	Stage-III	Stage-IV
PAS	++	++	++	++
AB	-	-	-	-
Hg-BPB	+++	+++	+++	+++
SBB	++	++	++	++
LF	+++	+++	+++	+++
BC	+++	+++	+++	+++

PAS = Periodic Acid Schiff's; AB = Alcian blue, Hg-BPB = Mercuric Bromophenol Blue; BC = Best Carmine, SBB = Sudan Black B in ethanol; LF = Luxol Fast, ++, +++ = Positive reactions, + = mildly positive reactions, -- = Negative reactions

In the present study, four successive stages in the development of oocytes in the process of oogenesis have been observed. It mainly tries to focus on the shape and size of oocytes, structure of nucleus, nucleoplasm and cytoplasm and histochemical nature of oocytes. However similar observations were reported by Kulkarni et.al.[7] in a fresh water leech Poecilobella viridis. Sareen et.al.[14] reported morphological and cytochemical aspects of vitellogenesis in Eisenia fetida. Similarly Anand[15] in Hirudo birmanica and Sagar[16] in Poecilobdella granulosa also found four developing stages during Oogenesis. Vijaya et.al.[12,13] reported presence of ovarian follicles of different developmental stages in the process of oogenesis and noticed presence of larger follicles at the periphery with many follicular cells and smaller primary follicles at the centre.

In the E.kinneari oocytes of ovary and spermatic follicles of testis revealed negative reaction with AB showed absence of mucopolysaccharides, mild reactions with PAS and SBB,while moderate reactions with Hg-BPB, LF and BC showed less quantity of carbohydrates, glycogen, and sufficient quantity of protein, lipids and phospholipids as reported by Lakhani [17,18.] Pastison[19] in Hirudo, Anderson and curgy[20] in Lumbricus reported occurrence of RNA synthesis during early stages of spermatogenesis, Protein synthesis has been studied in Hirudo (Pastison) and reported significant difference in the rate of orginine lysine, leucine and tryptophan during spermatogenesis.

Anand[15] studied on Indian leech Hirudo birmanica and reported presence of protein in oocytes and in sperm clusters, vitellogenic oocytes and mature spermatic clusters which showed positive results with periodic acid Schiff's reagent while immature sperm clusters and undifferentiated oocytes showed weak reaction with PAS, sperm clusters of testis and oocytes of ovary showed negative reaction with SBB, presence of protein and less quantity of carbohydrates in oocytes and spermatic follicles of Hirudo birmanica tally with present investigation. Similarly Bedre[21] in P.granulosa and Kulkarni[22] in Lampito mauritii biochemically estimated the presence of proteins, glycogen and lipids. Ramesh et.al[23] observed the presence of carbohydrates, proteins, lipids, phospholipids and nucleic acids in oocytes of onychophora horst. Sareen et.al[14] reported lipids, proteins and polysaccharides in oocytes of Eisenia fetida. The aforesaid results are more or less similar with present investigation.

Conclusion

Earthworms are important for protein production in animal feed industry, therefore high body weight of earthworms contribute towards higher protein production. Other important activity of earthworms is Vermicomposing. Earthworm's activity helps in the composting of disposed organic wastes from domestic and agricultural sources on behalf of their activities, it is necessary to provide favorable conditions to earthworms for long life span and time for reproductive maturity etc. It would be very helpful for breeding process of earthworms.

Looking to these utilities of earthworms Government and NGO's should take care of earthworms, because use of hazardous chemicals, pesticides, weedicides and insecticides affects population of earthworms adversely. These chemicals directly affect reproductive and nervous system is highly affected by these hazardous chemicals, resulting to generation abnormalities in them which reduce the worm population. It's our prime duty and responsibility to preserve Earthworm population as it is golden bough of agriculture industry of our country.

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