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HISTOCHEMICAL AND BIOCHEMICAL STUDIES ON THE OVARY IN THE BEETLE, CYBISTER TRIPUNCTATUS OL. (COLEOPTERA: DYTISCIDAE)

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ABSTRACT:
A pair of large ovaries are situated on either side of the alimentary canal occupying most of the abdominal cavity. Each ovary is composed of large number of ovarioles. The ovarioles are differentiated Antero posteriorly into four regions: terminal filament, germarium, vitellarium and pedicel. The vitellarium consists of a series of developing oocytes in linear fashion, each accompanied with a group of 15 nurse cells and thus representing polytropic – Meroistictype. The terminal oocyte undergo development through a series of consecutive 5 vitellogenic stages such as pre,early,mid,late and maturational stages.The deposition of yolk starts at the early vitellogenic stage dyring which transport of fine granules from follicular cells to the oocyte is well evident. During mid and late vitellogenic stages the oocyte grows in size and filled with the yolk bodies. The nusrse cells initially transport RNA to the vitellogenic oocyte and undergo degeneration prior to late vitelogenic stage. Thye histochemical and biochemical studies show protein lipid and carbohydrate composition of yolk material.

Keywords: *vitellogenesis, RNA, Yolk*

INTRODUCTION:

Female reproductive system and mechanism of oocyte development and vitellogenesis is of prime importance. The present work has, therefore, being undertaken to study female reproductive system in Cybister tripunctatus with special reference to the following aspects-

1. Morphology of female reproductive system;
2. Cytological changes occurring in developing oocytes, nurse cells andFollicular epithelial cells;
3. Histochemical demonstration of synthesis, accumulation and transport of DNA, RNA, protein, carbohydrate and lipid during oogenesis;
4. histochemical and biochemical analysis of the yolk material and secretory material of the colleterial gland andt thorough study of the process of vitellogenesis. Become surrounded by follicular epithelium and connected by the trophic cord. The young oocytes occur in previtellogenic stage during which the trophocytes transport material to the oocytes needed for their development. The nutritive cord collapse during vitellogenesis. The terminal oocytes undergo the process of vitellogenesis and finally the follicular epithelium secretes a vitelline membranes and the chorion. The number of ovarioles vary between species from one in s ome Scarabaeinae to about thousands in Meloproscarabaeus (Engelman, 1979; Raabe, 1986).

The base of each ovariole forms a pedical which unites and opens into a lateral oviduct. The lateral oviducts join to form a single median oviduct which is generally muscular and ectodermal in origin.

Cytological changes are observed in the cell lining of the oviducts. In Oncopltus fasciatus the cells of the anterior region of oviduct show secretary Activity, whereas, the cells of the posterior part help in the deposition of cuticle (Chen et al., 1962). The distal end of the common oviduct forms the gonopore which opens into the genital chamber. The genital chamber consists of the vagina which is present between the gonopore the external vulva. The vagina is covered whith thick muscle layer and lined by a cuticular intima. The genital chamber is modified forming the bursa copulatrix which stores the spermatophores. In Lepidoptera, the bursa is located on the 8th abdominal segment and is connected with the vagina by the seminal duct. In some butterflies e.g. Danus, tooth-like cuticular denticles are present in the Bursa copulatrix which help to cut-open the spermatophores. A pair of ectodermal glands are found associated with the genital chamber i.e. the spermathescae. The paired accessory glands or colleterial glands open into the vagina (Callahan and Chapin, 1960; Matsuda,1976). The morphology and number of spermathecae vary from species to species. In most insects, it is a single spherical organ and may have its distal end specialized for secretion to from the spermathecal

accessory gland. The secretion of spermathecal gland is mucoprotein or muco-polysaccharide and it is
An energy source to the spermatozoa stored in the spermatheca (Weaver and Edwards, 1990). Differentiation of spermatheca occurs in the late pupal stage and it is under the control of the hormone, 20-hydroxyecdysone (Raabe, 1986). In the Orthoptera, Chorthippuscurtipennis and Gomphocerusrufa, the
Differentiation is under the control of juvenile hormone (Kaulenas, 1992). In Rhodniusprolixus secretion of the median neurosecretory cells control the Differentiation of spermatheca (Davey,1985). The newly laid eggs in many insects are covered with a protective Coating. In Lepidoptera, the eggs are individually coated with a proteinaceous

METHOD AND MATERIAL:

The present work is carried out on the aquatic beetle, Cybister tripunctatus OL.
CLASSIFICATION (Richards and Davis 1977)Systematic position of the aquatic beetle, Cybister tripunctatus
OL is given below.
Class –Insecta
Subclass – Pterygota
Division –Endopterygota
Order – Coleoptera
Suborder –Adephaga
Family – Dytiscidae
Genus – Cybister
Species – tripunctatus

CHARECTERS

In aquatic beetles Cybistertripunctatus. Ol.,sexes are separate
And sexual dimorphism is well marked as the forelegs of male
Beetles show presence of adhesive pads, while such structures
Are absent in the females.
It possesses filliform antennae.
Hind legs are notatorial, functioning as swimming organs,
Flattened and fringed with large hairs.
Larvae are with long sickleshaped mandibles.
Last two abdominal segments along with abdominal lobes are Fringed with hairs.
Elytra store air beneath them.It is the source of oxygen which is supplied to tracheal system by last two pairs of abdominal spiracles, during diving in deep water.

SELECTION

Aquatic beetle, Cybistertripunctatus. OL, is selected for the present work because of the following reasons-

It is easily available, and commonly found in local ponds in all seasons.
It can easily be collected by fishing nets or hand nets in ample quantity.
It can be acclimatized under the laboratory conditions for a long duration due to their sturdy nature and their quick adaptability to new environment.
It can be maintained by feeding small fishes and crustaceans.
It is of convenient size to handle and easy for experimentation.

SOURCE

The aquatic carnivorous beetles were collected from the ponds located Pavani, Disti.Bhandara (MS). The beetles were reared in laboratory throughout the year to carry out the present studies.

REARING

The larvae and adult beetles were kept in well aerated aquarium in the laboratory. The mudy water and small stones having crevices were kept in aquarium to maintain natural condition. The small fishes were kept as a food of the beetles. The stones were kept to provide place for egg laying. Some times, they lay the eggs on the inner side of the wall of aquarium also. The aquarium was covered to prevent escaping of beetles from the aquarium. The fresh water was added for sufficient supply of oxygen.

The larvae and beetles were acclimatized and reared in laboratory under normal condition of photoperiod 12L : 12 D and 24ñC temperature. The mating occurred mostly during daytime. The mated female laid eggs in a capsule Like case which hatched within 3-4 days depending upon environmental conditions. The food was supplied once every day. The first instar larvae underwent two moults. The well developed third instar larvae were transferred into another aquarium. The larvae lastly constructed the pupal chambers in a soil. Newly emerged adults of both sexes were separated and kept into individual glass jars. The date and time of emergence of the adult beetles were recorded.

DISSECTON, FIXATION AND SECTIONING
The female reproductive organs dissected in insect Ringer'

Solution under stereoscopic binocular microscope. The organs were fixed in Bouin's fluid for 18-24 hrs for histology and in 6 to 12 hours in Carnoy's fixative for DNA, RNA. Protein and carbohydrate histochemistry. The fixed tissues were, dehydrated and embedded in paraffin wax at 60-62. The sections were cut at 4 and 10 um thickness on the microtome for histological and histochemical staining techniques respectively. For histochemistry of lipids, the ovaries, collecterial gland and spermathecal gland were dissected gently, fixed in Baker's calcium formal Fixative and sections of 10-15um thickness were cut on the cryocut (Leica, U.S.A.).

TECHNIQES

HISTOLOGICAL TECHNIQUES

Following histological techniques (Humason, 1962) were used-

1]Ehrlich's Haematoxylin-eosin (HE)

2] Heidenhain's Iron-Haematoxylin-orange G (FeH)

3] Mallory's triple stain.

BIOCHEMICAL TECHNIQES

In order to estimate total ovarian lipid, protein and carbohydrate concentration in the ovary and colleterial gland of the adult female beetles, they were sacrificed every day from emergence to the first ovipositio and ovaries and other structures were dissected out and processed for biochemical estimation.

PREPERATION OF EXTRACT

The ovaries and colleterial glands were removed gently after cutting the tergites of terminal region of the abdomen and immediately transferred to ice cold insect Ringer's solution. The fat bodies, trachea, nerves and other tissues were carefully removed and each ovary and colletrial gland were weighed to ± 1 m.g. Both the organs were thoroughly washed in ice cold Ringer's solution and then homogenized in ice cold citrate phosphate buffer (pH 6.8) and made upto 1 ml. The homogenate was centrifuged for 15 min. about 2000 revs/ min. The clear supernatant was stored under a drop of toluene at about 10$_0$C until required.

The following biochemical techniques were used for the estimation of total protein, lipid and carbohydrate concentration in the overies and colleterial Gland-

1] Total protein estimation by Layne's technique (Layne 1957). 2] Total lipid estimation by the Folch et al., method

(Folch et al., 1957) and 3] Total carbohydrate estimation by Hawk's method (Hawk et al: 1954).

EXPERIMENTAL DESIGN

The adult female beetles of known age viz; newly emerged (0-day),2,4,6,8 and 10-day old beetles, were taken out from each aquarium. The female reproductive organs were dissected out. The ovaries and a colleterial gland were separated, the weight was taken individualy and the organs were processed for various histological, histochemical and biochemical techniques described above.

OBSERVATIONS

THE FEMALE REPRODUCTIVE SYSTEM

MORPHOLOGY

The female reproductive system is well developed in the adult beetle ,Cybistertripunctatus (OL). It consists of (Fig.1) :

1. a pair of ovaries ;
2. a pair of lateral oviduct;
3. a common oviduct;
4. a vagina;
5. a spermatheca with a sp-ermathecal gland and
6. a colleterial gland.

The female reproductive organs are located in the abdominal cavity occupying the region comparising 1st to 8th abdominal segments. The reproductive organs are intermingled with the fat body and trachea. In the immature beetle, the ovaries are small laying ventral to the alimentary canal in the posterior 4th and 5th segments of the abdomen. In the matured beetles, the ovaries develop extensively and they occupy.

Each ovary is a large and oval in shape consisting about 20 to 25 ovarioles.Each ovariole is about 1.5 cm in length. An immature ovary measures about 8 to 12 mg and the fully matured one about 46 to 54 mg in weight. The ovaries are externally covred with a thin peritoneal sheath. The ovaries are attached anteriorly to the inner surface of the wall of the 1st abdominal segment by a suspensory ligament. They open posteriorly into the lateral oviducts. The lateral oviducts are short and tubular structures. They run latero-medially from the 6th to 7th abdominal segments. Both the lateral oviducts unite together forming common oviduct. The common oviduct is a large tubular and convoluted structure. The posterior part of the common oviduct is modified into the bulbus vagina. Large spindle shaped spermatheca is well-developed and located in the lateral region of the 4th to 6th abdominal segments. It opens ito the common oviduct at junction of the lateral oviducts by a fine elongated spermathecal duct. Terminally it bears a distinct tubular spermathecal gland.

The spermatheca is full formed in the matured female and differentiated into two region; the receptaculumseminis and laguna. A long thread-like, enormously coiled collaterial gland is present in the right lateral region of the abdominal cavity, below the ovary. The collaterial gland opens into the common oviduct.

HISTOLOGY

The ovarioles

The ovarioles are long, tubular structures (Fig.3). They are

Antero posteriorly differentiated into following four regions,

a terminal filament; a germarium; a vitellarium and a pedicel

The Terminal filament (Fig.)

Apical region of each ovariole contains stem line, the terminal filament. The terminal filament is anterior-most thread-like, slender, fine structure of the ovariole. The wall of the terminal filament is composed of a mass of small spherical cells with oval nuclei. The cells are almost devid of

Ch - Chorion
FE - Follicular epithelial
GM - Germarium
GV - Germinal vesicle'
NC - Nurse Cell
OC - Oocyte'
PC - Pedicel
TF - Terminal filament
VT - Vitellarium
VM - Vitelline membrane
YB - Yolk bodies

Fig.: Ovariole in Cybistertripunctatus (Diagrammatic)

cytoplasm. Internally a large lumen is present. Externally, the terminal filament is covered with a fine layer of tunica propria. A transverse septum lies in between the terminal filament and the germarium. The terminal filament of all ovarioles of an ovary unite together to form a thick cord, the suspensory ligament.

The germarium

(Fig.)

It is a small region clearly marked from the terminal filament by a transverse septum. It lies between the terminal filament and the vitellarium. It contains some cystoblasts, cystocysts and profollicular cells. The anterior Part of the germarium is exclusively filled with cystoblasts. The cystoblasts are spherical in shape and contain dense cytoplasm and large spherical nuclei at the centre. The cystoblasts produce cystocysts after undergoing mitosis. The cystocysts are closely packed in the posterior region of germarium. The posterior most region of germarium contains a germinal cyst forming an egg chamber.

The posterior region of the germarium in the matured beetles contains large number of trophocyts or nutritive cells, arranged in the several tiers along with a small basal oocyte, migrating to the vitellarium.

EXPLANATION OF FIGURES

Fig. HLS passing through early oocyte showing apical region of the ovariole differentiated into terminal filament and germarium IHE X 60

Fig. HLS passing through terminal filament IHE X 128

Fig. HLS passing through germarium IHE X 128

Fig. HLS passing through vitellarium of ovariole IHE X 16

Abbreviations:

TF - Terminal filament
GM - Germarium
TP - Oocyte
OOC - Trophocyte
TC - Nuclesa
NU - Pre-follicular cells

Discussion

The number of ovarioles in each ovary vary gratly greatly in Coleoptera. There is only one, ovariole per ovary in Scarabaeus and coprini (Heymons 1929, 1930), two per ovary in Pasalidae (Reyes Casillo and Richer, 1973), and Curuulionidae (Stein, 1847, Munro 1990-12, Bisell, 1937, Lenkova, 1949, Cram, 1958,Burke 1959, Vernier 1970, Garthe 1970, Stone et al 1971, 56 in Ctenicera (Zacharuk, 1958 a) and numerous in more than 329 species of beetles belonging To 45 families (Robgertson, 1961). In Cybistertripunctatus also 23-25 large number of ovarioles are observed in each ovary.

According to Stein (1847) the ovaries in Coleoptera are of three major Types -1. The ovary with pedicel of individual ovarioles, 2. The ovary with a common central pedicel and 3. The ovary with lateral pedicel, and in Cybister tripunctatus the ovaries represent the first type. Histology and development of the ovaries in Dytiscus is described by Korschelt (1886) and Demandt (1912) among Dytiscidae, while the present study is perhaps first on Cybister tripunctatus.

REFERANCE :

AGGRAWAL S.K., (1964), Histochemistry of vitellogenesis in the adult mealworm, Tenebrio molitor L. Cellule 64 : 371 – 379

AGGARWAL,S.K., (1967)

Morphological and histochemical studies on oogenesis in Callosobrunchusanalisfabr. (Bruchidae : Coleoptera)
J.Morph. 122 : 10 -33

AKOPYAN, L.A. AND MEGAKYAN Y.U.A. (1984)
The oogenesis of Ararat Cochineal. Changes in trophocyte ultrastructure during the follicular period of ovariole development Tistologiya 26 (12) : 1391 -1395

AKUTSU, S. AND YOSHITAKE, N., (1977)
Electron microscope observation on the vitelline membrane formation in the silkworm, Bombyx mori, L. J. Seri. Jpn. 46 : 509- 514

AMBROSIO, I. AND SCHEDL , P., (1984)
Gene expressing during Drosophila melanogaster Oogenesis ; analysis by in situ hybridization to tissue Selections. Dev. Biol. 105 : 80 – 93

ANDERSON, E. (1964)
Oocyte differentiation and vitellogenesis in the cock roach periplaneta Americana J. Cell .Biol. 20 : 131 -155.

ANDERSON ,L.M. AND W.H. TELFER , (1970)
Trypan blue inhibition of yolk depostion due to the follicle cell function in Cercopia moth, J. Embryol23 : 35 -53

ANDERSON, (1974)
Comprehensive aspects of ultrastructure of female gamete. Int. Rev. Cytol .4 : 1 - 64

ANDREW , R.J. AND TEMBHARE ,D.B. (1995)
Ultra structural post oviposition changes in egg chorion of The dragonfly Xyxommapetiolatum Rambur (Odonata :Libellulidae) Int. J. Insect Morphol and Embryol24 : 235 -238.

BAILEY, P. AND SHIPP, E. (1970)
Corpus allatum size and ovarian environment in inrradiated Cucumberfly, Dacuscumis J. Insect physiol., 16 : 1293 -1299

BAKER, J.R. (1946)
The histochemical recognition of lipinc quart J. Micro . Sci. 87 : 441 – 470.

BARDE, S. P. AND SHINDE , S.L. (1984)
Histomorphological correlates of the secretory activity in Parsintercerbralis and corpora allata with imposed virginity Normal reproductive phases of female Dinetus indicus Biviguanam, 10 : 157 -160

BAST,R. E. AND TELFER W.H. (1976)
Follicle cell protein synthesis and its contrtibutionto the yolk of the Cercopia moth oocytes. Devel .Biol . 52(1) : 83-97.

BECKERS (1956,b)
Revision of Nearctic species of Agrotis. Canadian Entomologist 88 : 1- 101

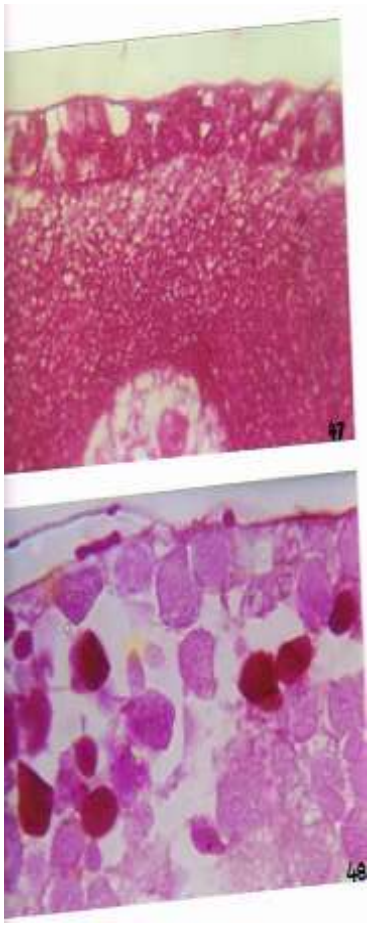
BEAMS, H.W., AND KESSEL, R. G. (1969)
Synthesis and deposition of oocyte envelopes (Vitellinge membrane, chorion) and the uptake of yolk in dragonfly (odanota :Aeschnidae) J. Cell Sci., 4 : 241 -264

BEENAKKEERS, A.M .TH., CHINO, H. LAW , J.H. (1998)
Lipohorine nomenclature, Insect Biochem18 : 1-2

BELLES , X., CASSIER , P., CERDA , S. , PASCUAL, NO., ANDRE, M., ROSSO, Y., AND PIULACHS, M.D., (1993)
Induction of choriogensis by 20 – hydroxyecdsone in the German coclroach Tissue cel .25 : 195-206

BERBEET, R.C., AND HELMS, T.J., (1972)
Comparitive anatomy and histology of selected system in Larval and adult Phyllophagaanxia(Coleoptera : Scarabaeidae). Ann .Entomol . Soc. Amer ., 65 (5) : 1026 -1053

BERRY, Y.S. , (1982)
Maternal direction of oogenesis and earl y embryogenesis in Insects. Annu. Rev. Entomol27 : 205 -227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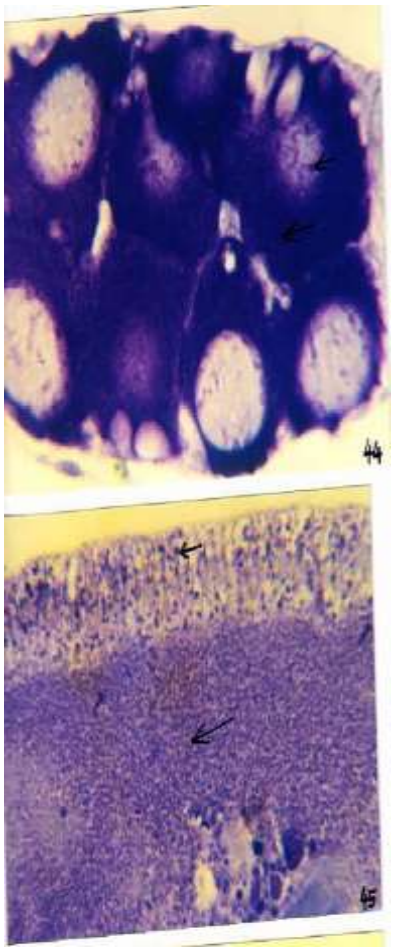
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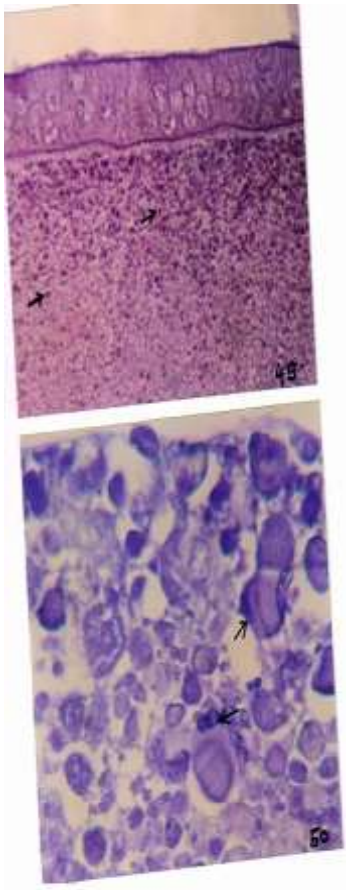
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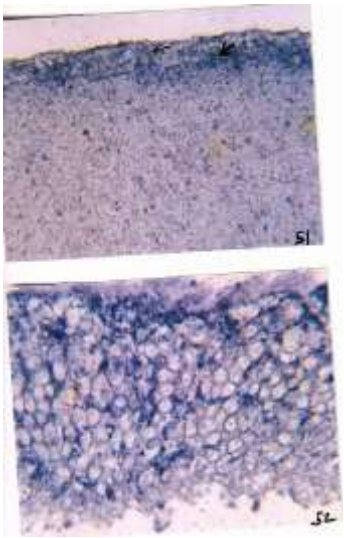
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