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STUDIES ON DEVELOPMENTAL STAGES OF SPERMATOGENESIS AND EFFECT OF JH III AND β-ECDYSONE ON SPERMATOGENESIS IN THE AQUATIC BEETLE, CYBISTER TRIPUNCTATUS (OL) (COLEOPTERA: DYTISCIDAE)

A. ZAHERA* AND D. B. TEMBHARE
Department of Zoology,
Rashtrasant Tukadoji Maharaj Nagpur University, Campus, Nagpur - 440033, INDIA.
e-mail: dr.zahera.16@gmail.com

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***Corresponding author**

ABSTRACT
The testis in *Cybister* tripunctatus is composed of a long coiled single follicle compactly filled with cysts. The follicle consisting gametes undergoing various stages of spermatogenesis from apically situated spermatogonia to the distally located spermatozoa can be seen distinctly. The process of spermatogenesis in dytistid beetle, *Cybister tripunctatus* is found occurring vigorously in the adult from month of February to August. Consequent changes in apical cells are well evident in the follicle. Presence of all the stages of spermatogenesis in adult indicates sperm production throughout the life of adult. The present studies reveal inhibitory effect of topical application of JH III and stimulatory effect of β-ecdysone on the process of spermatogenesis.

INTRODUCTION

The physiological aspects of male reproductive system are extensively discussed in various insects (Highnam, 1964;Davey, 1965;Engelmann, 1970; Adiyogi and Adiyogi, 1974; Happ, 1992 and Leather and Hardie, 1995). The studies on spermatogenesis have been carried out in large number of insects (Ratnakaran, 1971; a, b; Alexander and Chippendale, 1973; Slama, 1976; Numata and Hidaka, 1980; Lai-Fook, 1982;)Some workers, (Koolman *et al*., 1979; Loeb *et al*., 1984; Schimizu *et al*., 1985; Saxena *et al*., 1988) have noticed the presence of ecdysteroids in the extract of testis and suggested the hormonal control of spermatogenesis particularly, the induction of cell division and spermiogenesis. The role of 20-hydroxyecdysone in promoting spermatogenesis has been well documented in *Bombyx mori* (Takeuchi, 1969), *Chilo supperssalis* (Yagi *et al*., 1969), *Hyalophora cecropia* (Kambysellis and Williams, 1971), *Ephestia kuhniella* (Nowock, 1972, 1973), *Monema flavescens* (Takeda, 1972), *Memestra brassicae*, *Spodoptera litura* (Fukushima and Yagi, 1975), *Drosophila hydei* (Rungger- Brandle, 1976). In *Periplaneta*, removal of endocrine glands, testis transplantation and hormone injection showed that ecdysone accelerated testis development but JH inhibited it (Blaine and Dixon, 1976).In order to enrich our knowledge regarding physiology of male reproductive system in the aquatic beetle, *Cybister tripunctatus*, extensive studies were undertaken to elucidate especially, the structure, development and activity of the testis and male accessory glands with prime impetus to explore hormonal regulation of various reproductive mechanisms (Zahera, 2005). The findings pertaining to the effect of juvenile hormone (JH III) and β-ecdysone (βE) on male accessory glands (Zahera and Tembhare, 2015) was reported earlier while the present paper deals with the effect of JH III and β-ecdysone on spermatogenesis in the aquatic beetle, *Cybister tripunctatus*

MATERIALS AND METHODS

Histological methods

Large number of water beetles were collected from the local water bodies and were acclimatized in the laboratory. The testes were dissected from the adults in insect Ringer's saline and fixed in aqueous Bouin's fluid for 24 hr at the room temperature. The testes were washed thoroughly in the distilled water, dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax at 60- 62ºC. The 4 µm thick serial sections were cut and affixed to the Mayer's albumenized slides and stained with-

Ehrlich's Heamatoxylin eosin and Heidenhain's Iron heamatoxylin eosin. (Tembhare, 2008)

Effect of JH III and β-ecdysone

The treatment of JHIII and β-ecdysone was given topically to the three groups of the newly emerged male beetles in the

month of February. The 10 mg of JH III and 1mg of β-ecdysone (Sigma, USA) dissolved in 1ml cold acetone and 2μl were applied topically with the help of Hamilton's CR-700 constant rate syringe (USA) on the abdomen of the male beetles. The beetles of the first group were treated with acetone (vehicle for JH III and β-ecdysone) and served as the control insects while the second group with JHIII and third with β-ecdysone. The testes were dissected gently from 6-10 experimental and equal number of control insects after 1hr, 2hr, 4hr and 6hr intervals. The effects were studied on spermatogenesis in histological preparations of the testis.

RESULTS

Development of Testis and Spermatogenesis

Most of the development of the testis occurs in the adult stage in aquatic beetle, *Cybister tripunctatus*. During the present study, the developmental stages of spermatogenesis have been studied in the adults emerging in the month of February following the subsequent months.

The testis of adult *Cybister tripunctatus,* is unifollicular internally filled with number of maturing cysts exhibiting different stages of spermatogenesis (Fig. 1). The germ cells during spermatogenesis show cytological changes from one stage to another. The spermatogonia aggregate and clump into the cysts in which later on, divisions occur resulting in the formation of primary spermatocytes. The spermatocytes increase in number and become closely packed in the cysts. They increase in size, become oval or spherical in shape and the cysts thereafter, move towards a center of the follicle. In the months of February to May, the testis follicle showed spermatogonial cells arranged very compactly into the cysts in the anterior most part of the follicle. Each cyst spherical in shape is filled with about 90-100% spermatogonia showing lightly stained cytoplasm with the nuclei occupying a very small area and spermatocytes showing presence of darkly stained fibrous chromatin like chromosomes and nucleoli in the nuclei. (Figs.2, 3, 4 and 5). Their nuclei contain dense eccentric clumps of chromatin material. During the month of June the cysts were filled with 75-80% spermatids and spermatozoa with a fall in population of spermatogonia about 20-25 % of the cysts. The posterior part of the testis showed large number of cysts containing the stages of transformation of spermatids into spermatozoa (Fig. 6). During the month of July the cysts are filled with 60% spermatozoa and 10% spermatids. The spermatids differentiating into spermatozoa are well evident. A large number of sperm bundles are seen where the heads are fused together and the tails are free. Thereafter, during the month of August, the posterior cysts are heavily filled with the spermatozoa i.e. about 50 , while the anterior cysts are packed with about 50% the primary spermatogonia, secondary spermatogonia, primary spermatocytes suggesting completion of spermatogenesis. (Fig. 13). Concomitant changes in cell and nuclear diameter of the apical cells during spermatogenesis can be seen predominantly (Table 1) during subsequent months from February to August (Figs. 7 and 8). They are almost lost after formation of sperm bundles.

Effect of JH III and β-ecdysone

Control insects

In the control insects, the testis is filled with large number of cysts from apical to distal end. Most of the cysts are filled with spermatogonia and spermatocytes while only few posterior cysts contain the spermatids and spermatozoa .

Effect of JHIII

After 1hr, 2hr, 4hr and 6hr treatment of JH III, there was no change in the cysts containing gametes undergoing various stages of spermatogenesis than that in the control insects. Most of the cysts are filled with early spermatogenic stages such as Primary Spermatogonia, Secondary Spermatogonia and Secondary Spermatocyte similar to that in the control insects (Fig. 9).

Effect of β-ecdysone

Effect of β-ecdysone after 1 hr, 2hr, 4hr and 6hr of treatment shows enhanced spermatogenic activity in various cysts showing spermatogonia, spermatocytes, spermatids and spermatozoa. Transformation of spermatids into spermatozoa is occurring vigorously. Large numbers of cysts filled with newly formed sperm bundles are well evident. The apical cells and secretory droplets adjacent to spermatogenic stages are well evident (Fig. 10, 11 and 12).

DISCUSSION

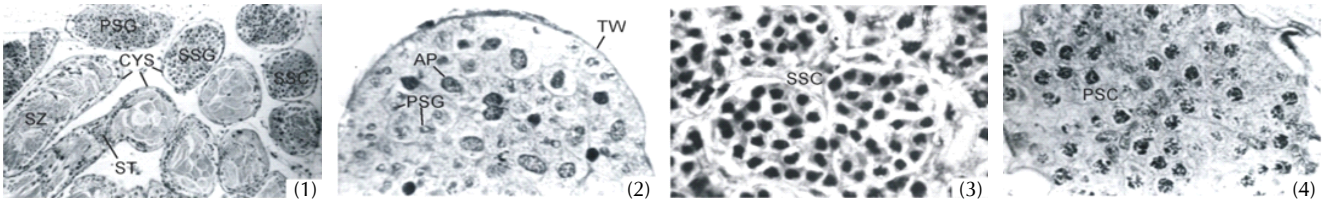
In the adult *Cybister tripunctatus,* the number of the cysts containing spermatogonia and spermatocytes decreases gradually during subsequent months. Similar to that noticed in a large number of Lepidoptera in pupal stage (Chaudhury and Raun, 1966; Holt and North, 1970). Reduction in the number of early spermatogenic stages and subsequent increase in late spermatogenic stages (spermatids and spermatozoa) occurs gradually from the month of February to August. Nevertheless, a stable spermatocyte pool is maintained through the continuous spermatogonial mitotic cycles (Lima De Faria and Nordquist, 1962). In *Cybister tripunctatus* all the stages of spermatogenesis with variation in cell and nuclear diameter of the apical cells during spermatogenesis show resemblance with the stages of spermatogenesis in the adult beetles, *Lytta nuttali* (Gerber *et al*., 1971), *Tenebrio molitor* (Gadzama *et al*., 1977), *Dytiscus marginalis* (Brelands and Simmons, 1970) and *Lampyris noctiluca*, (Balles *et al*., 2004), indicating that sperms are produced throughout the life of the adult.

Wigglesworth (1936) was the first to deliver his remarks that the matured sperm can be produced in the absence of JH in *Rhodnius prolixus* and JH is not necessary for spermatogenesis. Thereafter, Girardie and Vogel (1966), Cantacuzene (1967) and Foster (1967) supported the view of Wigglesworth. Slama *et al*. (1974) however, believed that the effect of corpus allatum hormone on the male reproductive system is limited only to stimulation of differentiation and function of accessory glands.

Studies on JH III and β-ecdysone showed strong stimulatory effect of JH III and inhibitory effect of β-ecdysone on the secretory activity of male accessory gland in the tasar silkworm, *Antheraea mylitta* (Pendam and Tembhare, 2005) in seminal vesicle secretions in the tasar silkworm, *Antheraea mylitta*

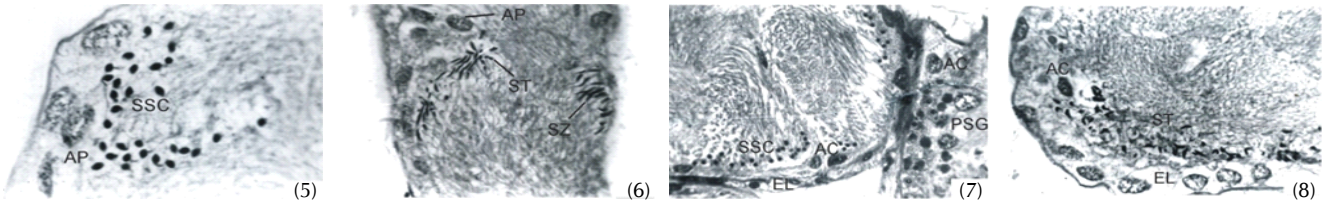
Table 1: Cell and nuclear diameter of the apical cells

Cell diameter (μm)						Nuclear diameter (μm)					
PSG	SSG	PSC	SSC	ST	SZ	PSG	SSG	PSC	SSC	ST	SZ
15.58 ±0.63	17.63 ±0.35	16.4 ±0.65	16.4 ±0.82	22.14 ±1.05	16.4 ±0.53	2.1 ±0.01	4.1 ±0.04	2.05 ±0.02	4.1 ±0.01	6.4 ±0.01	4.1 ±0.05



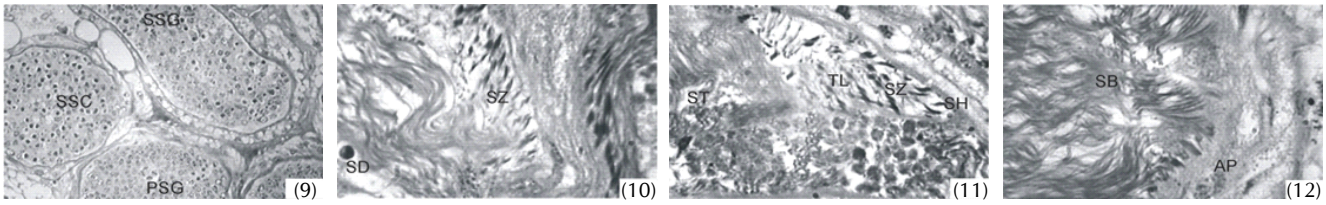
Abbreviation: SZ - Spermatozoa, ST - Spermatid, PSG - Primary spermatogonia, SSG - Secondary spermatogonia, SSC - Secondary spermatocytes, AP - Apical cell, TW - Testis wall, PSC - Primary spermatocycle, CYS - Cyst.

Figures 1-4: Longitudinal section of testis showing spermatogenic stages. FeH, Cysts containing different stages of spermatogenesis (X 50), section showing primary spermatogonia. (X 400), section showing primary spermatocytes (X 400)



Abbreviation: Ac - Apical cell, EL - Epithelial layer, SZ - Spermatoza, ST - Spermatid, PSG - Primary spermatogonia, SSC - Secondary spermatocytes, AP - Apical cell, TW - Testis wall

Figures 5-8: Longitudinal section of testis showing spermatogenic stages. FeH, section showing secondary spermatocytes (X 100), Setions showing transfomation of spermatids in to spermatozoa (X 600), Section, showing apical cell near SSC and PSG(X 200), Section showing darkly stained apical cell near spermatid (X 320)



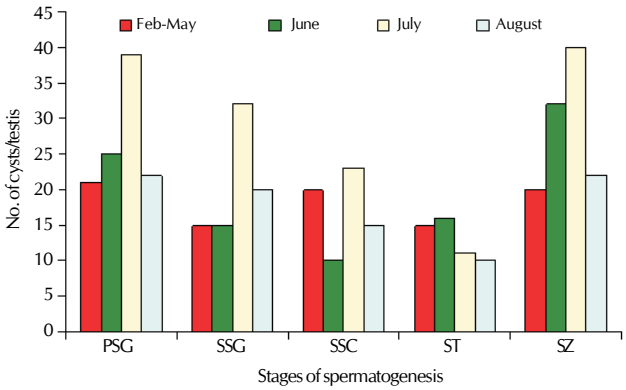
Abbreviation: AP - Apical cell, TL - Sperm tail, SH - Sperm head, SZ - Spermatozoa, ST - Spermatid, PSG - Primary spermatogonia, SSG - Secondary spermatogonia, SSC - Secondary spermatocytes, SD - Scretory droplets, SB - sperm Bundle.

Figures 9-12: Section of testis after JH lll and β-ecdysone treatment. FeH,Section showing PSG,SSG and SSC(X 150),Section showing scretory droplets and spermatozoa (X600), Abbr, AP-apical cell, TL-sperm tail, SH-sperm head, SZ-spermatozoa, ST-spermatid, PSG-primary spermatogonia, SSG-secondary spermatocytes, SD-secretory droplets, SB sperm Bundle

(Pendam and Tembhare, 2013) and male accessory gland secretions in aquatic beetle , *Cybister tripunctatus*(Zahera and Tembhare, 2015).

In *Cybister tripunctatus* the process of spermatogenesis in the testis of the experimental and control insects is found almost similar suggesting no significant effect on spermatogenesis after the treatment of JH III.

On the other hand, 20- hydroxyecdysone promoting spermatogenesis in late larval life or during the pupal stage has been well documented in *Bombyx mori* (Takeuchi, 1969), *Chilo suppressalis* (Yagi *et al.,* 1969), *Hydophora cecropia* (Kambysellis and Williams 1971), *Ephestia kuhniella* (Nowock, 1973), *Monema flavescens* (Takeda 1972), *Mamestra brassicae* and *Spodoptera litura* (Fukushima and Yagi 1975), and *Rhodnius* (Dumser and Davey 1975; Bodnaryk, 1986) *Heliothis virescens* (Loeb *et al.,* 1982; 1984; 1985; 1986; a,



PSG - Primary spermatogonia, SSG - Secondary spermatogonia, SSC - Secondary spermatocyte, ST - Spermatid, SZ - Spermatozoa.

Figure 13: Number of cysts containing gametes in a month

b, 1987; 1988) *Dysdercus koenigii* (Sexena and Tikku, 1989; 1991) *Manduca sexta* (Friedlander and Reynolds, 1992) and *Antheraea mylitta* (Tembhare and Pendam, 2005). Happ (1992) in his review strongly supported the regulation of spermatogenesis by 20- hydroxyecdysone in insects. Secretion of 20 - hydroxyecdysone as the male gonadal hormone by apical cells in the testis of some insects, *Calliphora vicina, Dysdercus intermedius* (Koolman *et al.,* 1979; Loeb *et al.,* 1982), *Heliothis* virescens and *Gryllus bimaculatus* (Hoffmann *et al.,* 1982) has been investigated thoroughly. It is experimentally proved that the 20–hydroxyecdysone promotes spermatogenesis in insects, (Dumser and Davey, 1974, 1975; Takeuchi, 1969; Yagi *et al.,* 1969; Nowock, 1973; Takeda, 1972; Fukushima and Yagi, 1975; Rungger –Brandle, 1976). In *Cybister tripunctatus* occurrence of well-defined apical cells in the testis lobule and activity during differentiation of male gametes is well evident suggesting them as the source of male gonadal hormone.

On the basis of the present experimental studies it can be concluded that the juvenile hormone JH III exerts no significant effect on the process of spermatogenesis, while the β-ecdysone stimulates spermatogenesis causing higher rate of production of spermatocytes and spermatozoa in the testis suggesting stimulatory effect of ecdysone on spermatogenesis in aquatic beetle, *Cybister tripunctatus*.

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