Effect of precocene on development of ovarian follicles in flesh fly, *Sarcophaga ruficornis* F.

Krishna Kumar * & Irfan Ahmad Khan

Department of Zoology, University of Allahabad, Allahabad 211 002, India

Received 2 April 2003; revised 2 September 2003

Administration of precocene II (6,7-dimethoxy-2,2-dimethyl chromene) to freshly emerged virgin female flies of *S. ruficornis* adversely affected the development and differentiation of ovarian follicles leading to a number of morphological abnormalities. Precocene treatment resulted into suppression of development of egg chamber, differentiation of follicular epithelium, degeneration of nurse cells, growth of oocyte and uptake of yolk granules by oocytes. The results suggest that precocene induced effects are due to deficiency of juvenile hormone.

**Keywords**: Flesh fly, Ovarian follicles, Precocene, *Sarcophaga ruficornis*

Juvenile hormone (JH) plays an important role in controlling oogenesis in many insects. There exists a direct correlation between corpus allatum activity and the development of oocyte. In cyclorrhaphous Diptera such as *Phormia regina* and *Sarcophaga bullata*, removal of corpora allata shortly after emergence results into retardation or prevention of oogenesis. Application of juvenile hormone to sugar fed *Phormia regina* flies which is an anautogenous species leads to full ovarian development showing that juvenile hormone causes autogeny in this species. Juvenile hormone also controls uptake of vitellogenin by oocytes in Dipteran insects like *Drosophila melanogaster* and *Phormia regina*.

Anti-JH compound, precocene, selectively destroys corpora allata of insects resulting into JH deficiency. Within the corpora allata, precocene is converted into precocene 3,4-epoxide and the reactive epoxide either undergoes hydration to from precocene 3,4-dihydrodiol or alkylates cellular components leading to damage of the glands. Therefore, all the juvenile hormone dependent processes can be evaluated by the application of precocene. In Diptera, application of precocenes produces varied effects. Precocene does not exhibit specific antigonadotropic effect in *Aedes aegypti*, inhibits vitellogenesis in *Drosophila melanogaster* and produces sterility in female offsprings of treated females of *Glossina morsitans*. The present communication describes the effect of precocene II on the development of ovarian follicles in the flesh fly *Sarcophaga ruficornis* F. (Diptera: Sarcophagidae).

**Materials and Methods**

Precocene II (6,7 dimethoxy-2,2 dimethyl chromene; ALD 19491-3, Aldrich Chemical Co., U.S.A.) was dissolved and diluted in acetone to obtain different concentrations in 1 µl acetone. Freshly emerged adult virgin female flies were anaesthetized with ether to facilitate easy handling and then topically treated with different concentrations of precocene viz., 75, 100, 150 and 200 µg in 1 µl acetone applied on the ventral surface of abdomen with the help of a Hamilton syringe. Control flies were similarly treated with 1 µl of pure acetone only. After treatment, the treated flies were reared with normal males. The flies were given sugar cubes, water and fresh pieces of goat’s liver for feeding. The latter constituted standard protein source and was also used for larviposition. When larviposition had occurred in control flies on 10/11 day, treated flies (in which larviposition did not occur) were sacrificed and dissected in insect Ringer’s solution to take out their ovaries. For histological studies, ovaries of normal and treated flies were fixed in Carnoy’s fluid, embedded in paraffin, sectioned and stained in Delafiel’s haematoxylin and counterstained in eosin. An ocular micrometer scale was used for measuring length and width of egg chamber, oocyte and area occupied by nurse cells and their volume was calculated by the formula: $V = 0.5236 W^2L$, where $V = \text{volume}$, $W = \text{maximum width}$, $L = \text{maximum length}$. 

---

*For correspondence:
Phone: 0532-2605903 (R)
E-mail: kksamaruni@rediffmail.com*
Results

In Sarcophaga ruficornis, two ovaries are found. Each ovary is polytrophic containing 20-45 ovarioles (Fig. 1). Each ovariole has a terminal filament, germarium and vitellarium. The terminal filament is a short prolongation of peritoneal layer whereas germarium contains group of cells which produce egg chambers. The vitellarium contains an average of 2 egg chambers, all in pre-yolk stages. In freshly emerged adult flies, the ovaries are very thin and shrunken but subsequently become large and conspicuous due to rapid growth of terminal egg chamber.

When freshly emerged virgin females were treated with 200 μg precocene II, 100% mortality occurred and all the flies died within two days of emergence. Administration of 75, 100 and 150 μg precocene II to virgin females produced a number of morphological abnormalities in ovaries which were dissected out after larviposition in controls (10-11 days after emergence). On the basis of morphological abnormalities, the ovaries of precocene treated flies could be broadly classified into following grades:

I. Ovaries containing fully developed larvae but these were not laid in uterus and consequently larviposition did not occur (Fig. 2).

II. There was degeneration of follicles and the affected part turned black and membranous (Fig. 3).

III. The two ovaries fused together giving rise to a composite structure (Fig. 4).

IV. Ovaries with small and irregularly arranged follicles (Fig. 5) or both ovaries consisting of loose and highly scattered follicles (Fig. 6).

V. The two ovaries were unequal in size. The follicles were disorganised, detached and lying separately (Fig. 7).

VI. One ovary consisting of only two follicles whereas in other ovary, follicles were small and irregularly arranged (Fig. 8).

Ovaries showing morphological abnormalities of grades I-III were produced when 75 μg precocene II
was administered to freshly emerged females whereas grades IV-VI were formed at higher doses of 100 and 150 μg of precocene II.

The size and volume of egg chamber, oocyte and the nurse cells of control and treated flies are given in Table 1. Before larviposition (10/11 day), ovaries of normal control flies (day 8) contained fully developed ovarioles. The oocyte was fully mature and completely filled with yolk granules. The nurse cells were degenerating and their nuclei were shrunken and disintegrating. The follicular epithelium was also degenerating and the egg membranes were formed (Fig. 9). In flies treated with 75 μg precocene II, the follicular epithelium above the nurse cells was not visible whereas follicular epithelium surrounding the oocyte consisted of flat cells with distantly placed nuclei but without cell boundaries. Some epithelial cells were seen having been moved between the nurse

Table 1 - Size (length and width in μm) and volume (μm³) of egg chamber, oocyte and nurse cells in control and precocene treated flies
[Values of size are mean ± SE from 10 observations in each case]

<table>
<thead>
<tr>
<th>Dose (μg)</th>
<th>Egg Chamber</th>
<th>Oocyte</th>
<th>Nurse Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length</td>
<td>Width</td>
<td>Mean volume</td>
</tr>
<tr>
<td>75</td>
<td>127.8±1.48†</td>
<td>38.4±0.44†</td>
<td>98671.77</td>
</tr>
<tr>
<td>100</td>
<td>49.4±0.82*</td>
<td>27.2±0.52*</td>
<td>19136.58</td>
</tr>
<tr>
<td>150</td>
<td>52.5±1.58*</td>
<td>25±0.48*</td>
<td>17082.45</td>
</tr>
<tr>
<td>Control</td>
<td>131.2±0.77</td>
<td>38.2±1.82</td>
<td>100244.41</td>
</tr>
</tbody>
</table>

P values: * <0.001; **<0.01; † >0.005; † † >0.10

Figs 5-8—(5-6)—Ovaries of precocene treated flies of grade IV; (7)—Ovaries of precocene treated fly of grade V and (8)—Ovaries of precocene treated fly of grade VI [Figs 5-8; Bar =0.02 mm]
cells and oocyte (arrow) and ring canal and some space were observed between these cells and oocyte (arrow) and oocyte contained yolk granules (Fig. 10). Deposition of egg membrane had taken place towards the anterior side of the egg chamber. Nurse cells nuclei were degenerating and this was more prominent in cells located towards anterior side as compared to those found near the oocyte. However, the volume of nurse cells was still greater than that found in normal untreated flies (Table 1; Fig. 10).

In flies treated with 100 J.µg precocene II, the follicular epithelium consisted of normal cuboidal cells around the oocyte and squamous above the nurse cells. No intercellular spaces or bridges were observed. However, a few intercellular spaces were visible towards the posterior extremity of oocyte. The nurse cell nuclei were prominent and those situated close to oocyte were larger as compared to those found at the anterior end. Some dark and bigger yolk granules were seen in the oocyte. Egg membranes were not visible (Fig. 11).

In flies treated with 150 µg precocene II, the follicular epithelial cells consisted of round nuclei with inconspicuous boundaries and there was great vacuolation in epithelium. The nuclei of follicular epithelial cells were seen intruding into the ooplasm.

Figs 9-12 — Photomicrograph of egg chamber of normal control fly (8 days old) (10)—Photomicrograph of egg chamber of fly treated with 75 µg precocene II; (11)—Photomicrograph of egg chamber of fly treated with 100 µg precocene II and (12)—Photomicrograph of egg chamber of fly treated with 150 µg precocene II [Figs 9-12; Bar=0.005 mm]
There was also vacuolation in nurse cells and ooplasm. Some deposition of yolk granules had taken place. Egg membranes were not developed (Fig. 12). When egg chambers of treated flies were examined in pre yolk stage (day 2 and 3), the follicular epithelium was irregular and highly vacuolated, the nuclei were visible but cell boundaries were absent (Figs 13 and 14).

Discussion
The anti-JH compound, precocene, suppresses the development and maturation of ovaries in hemimetabolous insects such as Diploptera punctata15, Oncopeltus fasciatus21 and Nilaparvata lugens2. In holometabolous insects, precocene administration results into various abnormalities in ovaries of lepidopteran, Corcyra cephalonica23 and Spodoptera mauritia24 whereas terminal oocyte development is inhibited in dipteran flies, Phormia regina12 and Drosophila melanogaster25. The flesh fly, Sarcophaga ruficornis, appears susceptible to precocene as ovaries of precocene treated flies fail to develop normally and exhibit a series of morphological abnormalities like degeneration of follicles and unusual fusion of two ovaries at lower concentration whereas at higher concentration, the growth of follicles is inhibited apart from reduction in number and irregularity in arrangement. Moreover, in cases where ovaries are developed, the larviposition is prevented.

Administration of precocene II to freshly emerged females of S. ruficornis suppresses the development of egg chamber and growth of oocyte in a dose-dependent manner. With the increase in dose of precocene II, volume of egg chamber and oocyte decreases as compared to that of control untreated flies. Similarly degeneration of nurse cells is adversely affected in precocene treated flies as the volume of nurse cells is greater in treated insects as compared to that found in controls. Precocene treatment also inhibits the differentiation of follicular epithelium in S. ruficornis. During normal development, the follicular epithelium consists of cuboidal cells (day 2) but subsequently due to increase in size these cells become columnar. Later the follicular epithelium around the nurse cells becomes thin and the follicle cells enveloping the oocyte become columnar with wide intercellular spaces. As the oocyte matures, the follicle cells surrounding the oocyte represent mere debris of weakly staining material and deposition of egg membranes is completed26. As compared to columnar follicle cells with wide intercellular spaces in normal flies, in treated flies, the follicular epithelium consists of either flat or cuboidal cells or epithelium consisting of round nuclei without cell boundaries and showing high degree of vacuolation. Intercellular spaces were also not visible in follicular epithelium of treated insects and consequently vitellogenesis was suppressed because yolk proteins are supposed to enter the oocyte via the intercellular spaces27,28. Koeppe et al.29 have shown that juvenile hormone induces structural changes in the follicular epithelium; the appearance of channels between adjacent follicle cells and of spaces between the follicular epithelium and the maturing oocyte; an increase in follicle cell size; and an enlarged nucleus within each follicle cell.

Figs 13 & 14—(13)—Photomicrograph of egg chamber (2 days old) of fly treated with 150 µg precocene II and (14)—Photomicrograph of egg chamber (3 days old) of fly treated with 150 µg precocene II. Figs 13, 14; Bar = 0.005 mm. [Abbreviations: C = Chorion; FE = Follicular epithelium; NCN = Nurse cell nucleus; OC = Oocyte; OCN = Oocyte nucleus; YG = Yolk granules]
Therefore, suppression of differentiation of follicular epithelium in precocene treated female flies of *S. ruficornis* appears due to JH deficiency. It has been earlier observed that in *S. ruficornis*, precocene treatment results into precocious metamorphosis due to juvenile hormone deficiency and the precocene induced effects can be abolished by application of juvenile hormone. The suppression of differentiation of follicular epithelium is more severe at higher doses of 100 and 150 μg of precocene. However, at a lower dose of 75 μg, some follicle cells were seen to have been moved in between the nurse cells and oocyte and ring canal and space were observed between these cells and oocyte. Egg membrane was also formed at some places such as towards the anterior end of the oocyte at a dose of 75 μg but its formation was completely prevented at a higher dose of 100 and 150 μg.

In precocene treated flies, reduction in the volume of oocyte as compared to that found in controls, is apparently due to inhibition of uptake of yolk granules by the oocyte. In Dipteran flies, the uptake of vitellogenin by oocytes or vitellogenesis is a juvenile hormone dependent process. In *Drosophila melanogaster*, the development of vitellogenic oocyte was inhibited by treatment with precocene I and II and the volume of corpus allatum in the treated insects was significantly decreased. Similarly in blow fly, *Phormia regina*, oocyte development was significantly retarded as a result of treatment of precocene II and application of a juvenile hormone analogue, methoprene, reversed the precocene inhibited oocyte development. In *Phormia regina*, it was also observed that corpus allatum of treated flies occupied significantly small volume and release of juvenile hormone by the glands was significantly reduced but not completely stopped. In *Sarcophaga ruficornis*, it has been earlier observed that precocene induced developmental abnormalities were due to juvenile hormone deficiency and exogenous application of juvenile hormone to precocene treated larvae reversed the effect of precocene. Therefore, it appears that precocene induced inhibition of development of vitellogenic oocyte of *S. ruficornis* is due to deficiency of juvenile hormone. In *D. melanogaster*, precocene directly acts on the corpus allatum since precocene treatment inhibits oocyte development in decapitated females and, therefore, precocene action is not mediated by the brain.

**Acknowledgement**

The authors are grateful to late Prof. U.S. Srivastava for inspiration, valuable suggestions and helpful criticisms. Financial assistance from UGC, New Delhi, is acknowledged.

**References**

17 Kelly T J & Fuchs M S, Precocene is not a specific antigonadotropic agent in adult female *Aedes aegypti*, *Physiol Ent*, 3 (1978) 297.


