STIMULATION OF OOGENESIS IN THE FRESHWATER PRAWN, MACROBRACHIUM LAMERRII BY PROSTAGLANDIN E₂ AND FOLLICLE STIMULATING HORMONE

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ABSTRACT
The effect of prostaglandin E₂ and follicle stimulating hormone on oogenesis was studied in the freshwater prawn, Macrobrachium lamermii. The ovaries of the base control and experimental control were small and white in colour and their gonosomatic indices were 1.06 ± 0.27 and 1.50 ± 0.24 respectively. Histological observations indicate that base control ovaries showed oogonia, primary oocytes and few primary vitellogenic oocytes. Primary vitellogenic oocytes with proliferating zone were noticed in experimental ovaries. In prostaglandin E₂ treated group the colour of the ovary changed from white to green and the gonosomatic index increased to 4.08 ± 0.6. Secondary vitellogenic I, II and few primary vitellogenic oocytes were observed.

The ovaries of FSH administered group were light green in colour and the gonosomatic index was 3.12 ± 0.40. Active proliferation of oocytes, few primary vitellogenic and secondary vitellogenic-I oocytes were observed.

INTRODUCTION
Prostaglandins are produced exclusively in the plasma membrane of cells and are derived mainly from arachidonic acid which is released from phospholipid by the action of phospholipases. Prostaglandins which are widely distributed in the animal kingdom (Christ and Van Dorp, 1972; Nomura and Ogita, 1976; and Ogita et al., 1978) exert profound effects on the reproductive system in mammals. Several workers demonstrated recently that prostaglandins have a role in ovulatory process of some mammalian species (Lemaire et al., 1973, 1975) and hen (Day and Nalbandov, 1977). Stacey and Panday (1975) showed that indomethacin blocks ovulation induced by HCG (Human chorionic gonadotropin) injection in goldfish, but exogenous prostaglandins overcome the indomethacin blockade. The positive effect of prostaglandin on induction of ovulation in a freshwater cat fish was demonstrated in vivo by Singh and Singh (1976). So far there is no work on the effect of prostaglandins on crustacean species, and hence it was decided to examine the influence of prostaglandin E₂ on the ovary of Macrobrachium lamermii.

Hormonal regulation of breeding has been found in all higher animal groups including crustaceans (Gilbert, 1963). In the breeding scheme, certain similarities can be found between Arthropoda and Vertebrata. Above all, the course of oogenesis is similar, but with regard to the mechanisms that control the maturation and functions of the gonads, these two animal groups differ considerably. The most important hormone responsible for the regulation of gonadal maturation in crustaceans is gonad-inhibiting hormone (GIH), while in vertebrates the gonadotropic hormones of the pituitary body stimulate the function of the gonads. The number of comparative studies of the reciprocal
action of hormones among vertebrate classes is fragmentary and there have not been any studies of this type concerning such an action between invertebrates and vertebrates.

Bomirski and Klok-Kawinska (1976) demonstrated that the human chorionic gonadotropin (HCG) has a stimulating effect on the oogenesis in the sand shrimp, Crangon crangon, regardless of the presence of the source of the endogenous GTH. Recently Sarojini et al., (1986) documented the response of estuarine crab, Scylla serrata ovaries to follicle stimulating hormone (FSH). The results indicate a possibility of controlling the function of the gonads in prawns by treating them with gonadotropic hormones produced by individuals belonging to a different phylum.

In order to further test this possibility, in the present investigation the effect of porcine hypophyseal gonadotropin, FSH, on the morphological and histological structure of the ovaries in the freshwater prawn, Macrobrachium lamerrii was studied.

**MATERIAL AND METHODS**

The test animals were immature female Macrobrachium lamerrii, collected from Paithan near Aurangabad. To preclude any variation in results, prawns of uniform size, gonadal development, apparently healthy non-ovigerous and intermoult (Stage-C) were selected for the experiments. The aquaria were aerated and animals were fed with soft green algae on every alternate day. The water was changed daily; all experiments were conducted at normal day-light illumination.

For hormone preparations, 10 mg of FSH and 2 mg of prostaglandin E₁ were dissolved separately in 1 ml of 1% ethanol and resulting solution was diluted up to 10 ml by adding distilled water. Final concentration of FSH preparation was 1 μg per 1 μl and prostaglandin preparation was 0.2 μg per 1 μl. Various doses of these hormones were tried and the selected doses and durations provided the maximum effect.

A total of eighty prawns were divided into four groups of 20 each. Group 1 served as base control, group 2 received 25 μl/prawn of 1% ethanol (Experimental control) whereas group 3 and 4 received 25 μl/prawn of prostaglandin E₁ and FSH respectively on 5, 10, 15 and 20th day through arthoridal membrane into the abdominal musculature.

Ovaries were dissected out and fixed in Bouin's fluid at the start of the experiment in the base control prawns and on the 21st day (24 hours after last injection) in remaining groups of prawns. Ovaries were dehydrated in graded series of alcohol, embedded in paraffin wax (58-60°C), sectioned at 8 μm, stained with Harris's Haematoxylin-eosin and mounted in DPX for histological scrutiny of oocyte stages.

**RESULTS**

**Base control ovary**

The ovaries were small, white in colour (which corresponds to immature stage), enveloped with a thin connective tissue layer and their gonosomatic index (GSI) was 1.06 ± 0.27 (Table 1). Ovaries showed

<table>
<thead>
<tr>
<th>Animal category</th>
<th>Gonosomatic Index (GSI)</th>
<th>Oocyte diameter (μm) ± S.D.</th>
<th>Colour of the ovary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base control</td>
<td>1.06 ± 0.27</td>
<td>68 ± 3.12</td>
<td>white</td>
</tr>
<tr>
<td>Experimental control</td>
<td>1.50 ± 0.24</td>
<td>115 ± 5.01</td>
<td>white</td>
</tr>
<tr>
<td>Prostaglandin E₁</td>
<td>4.08 ± 0.61</td>
<td>222 ± 6.15</td>
<td>green</td>
</tr>
<tr>
<td>FSH</td>
<td>3.12 ± 0.40</td>
<td>125 ± 5.26</td>
<td>light green</td>
</tr>
</tbody>
</table>
oogonia, primary oocytes and primary vitellogenic oocytes. These oocytes were small, few in number and possessed a compact nucleus with an invisible rim of cytoplasm. The proliferating zones were single, small and inactive. At this time the average size of the largest oocytes was about 68 ± 3.12 (Fig. 1) (Table 1).

Experimental control ovary

It did not differ much from the base control. The proliferating zone was active, the young oocytes were surrounded by a vitelline envelope. More primary vitellogenic oocytes and few secondary vitellogenic oocytes with a diameter of 115 ± 5.01 μm were observed in this group (Fig. 2) (Table 1).

Fig. 1. T.S. of ovary of base control showing oogonia, primary oocytes and primary vitellogenic oocytes. Hematoxylin-eosin. X 150.

Fig. 2. T.S. of ovary of the experimental control showing normal oocyte development. Hematoxylin-eosin. X 100.

Fig. 3. T.S. of ovary injected with prostaglandin F2 showing few primary vitellogenic and many secondary vitellogenic oocytes. Hematoxylin-eosin. X 100.

Fig. 4. T.S. of ovary injected with FSH showing active proliferation of oocytes and vitellogenic - I oocytes. Hematoxylin-eosin. X 250.

PC Follicle cells
N Nucleus
NU Nucleolus
OG Oogonia
PO Primary oocytes
PVO Primary vitellogenic oocytes
PZ Proliferating zone
SVO Secondary vitellogenic oocytes
YGR Yolk granules

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Prostaglandin E2 treated ovary

After treatment with prostaglandin E2, the colour of the ovary changed from white to green (which corresponds to matured stage) and the GSI increased to 4.08 ± 0.61. Histological observations revealed that nearly all the oocytes entered the phase of primary and secondary vitellogenesis. These vitellogenic oocytes were loaded with dense yolk globules and cell size increased significantly (+ 212 ± 6.15 μm) when compared with base control ovary and experimental control ovary. The nuclei of these oocytes were less compact, possessed a chromatin reticulum and one or two centrally or peripherally located nucleoli. Each oocyte was surrounded by numerous follicular cells (Fig. 3) (Table 1).

FSH treated ovary

The ovaries of FSH administered group were light green (which corresponds to maturing stage) in colour and GSI was 3.12 ± 0.40. Injection of FSH showed maximum proliferation of gonadal cells and rapid accumulation of yolk granules. Few primary and secondary vitellogenic oocytes and more oogonial cells were observed. The vitellogenic oocytes have well defined nucleus with one or two nucleoli. The ooplasmic material was filled with yolk granules and the oocytes measured 125 ± 5.26 FSH also increased the follicle cell number and size (Fig. 4) (Table 1).

DISCUSSION

In the present study, the prostaglandin E2 caused stimulatory effect on the oogenesis. The transformation of oogonia into oocytes, heavy yolk deposition and dark nucleus suggest that prostaglandin E2 enhanced vitellogenic activity in M. lamellii. Prostaglandins have been widely implicated in several physiological aspects of vertebrates (Aldridge et al., 1970; Hertelendy, 1972; Day and Nalbandov, 1977). In rats the injection of prostaglandin E2 directly into the brain ventricle stimulated gonadotropin secretion (Spies and Norman, 1973; Harms et al., 1974) and it has been suggested that prostaglandins stimulated the release of LHRH (Leutinizing hormone releasing hormone) and thereby gonadotropins.

In M. lamellii regarding the mode of action of prostaglandin E2 it can be suggested that injected steroid hormone as in the vertebrates where prostaglandins activate the steroid centres of the brain which in turn stimulate the ovary, releases gonadotropins (Spies and Norman, 1973; Harms et al., 1974). There is good evidence that prostaglandins may also participate as intracellular messengers of hormone action in smooth muscles (Hadley, 1984). In M. lamellii prostaglandin E2 may also be acting as a messenger of hormone action activating the ovary.

The effect of hypophyseal hormone, FSH on the histological structure of the ovaries is known relatively well in mammals and much less in other vertebrates and it is completely unknown in crustaceans.

FSH is responsible for complete development of the ovarian follicle in the female and is necessary for the final stage of spermatid maturation in male. FSH alone is sufficient for the complete development of the ovarian follicle. Several recent studies suggest that effects of FSH on the ovary are mediated primarily through the granulose cells. FSH is also capable of initiating granulose cell leutinization (Channing, 1972 and Morin, 1977). FSH but not LH, stimulates the conversion of testosterone to estradiol-17 B in cultured ovaries of hypophysectomised immature rats.

Treatment of crustaceans with FSH and LH (Zukowska, 1981) had a stimulating effect on oogenesis, brought about by a growth of the proliferating zones and transition of oogonia to meiotic oocytes. The stimulating effect on the latter phases of the growth of the vitellogenic oocytes appeared to be slight. The results corroborate with those obtained for FSH in M. lamellii.
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REFERENCES


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