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Role of vertebrate type of steroids in relation to ovarian maturation in mud crab, *Scylla serrata* (Forsskal, 1775)

Helen Sundara Bai, R. Kirubagaran*, and S. L. Sasikala*

Suganthi Devadason Marine Research Institute, 44, Beach Road, Tuticorin - 682 001, India, E mail: helenbio@gmail.com
** National Institute of Ocean Technology, Chennai - 601 302, India*

Abstract

The changes in the levels of vertebrate-type hormones estradiol-17β and progesterone in the haemolymph and ovary of the mud crab *Scylla serrata* in relation to different stages of ovarian maturation has been investigated. The histological study of the ovary of the crab of 250 ± 10 g average weight showed a large number of oogonial cells at stage I, oocytes with yolk granules at stage II, oocytes filled with yolk droplets at stage III and fully matured oocytes having ooplasm filled with yolk globules at stage IV. The radioimmuno assay carried out to quantify the 17β- estradiol and progesterone in the haemolymph indicated that the estradiol is nondeductible in the haemolymph in stage I. And it showed an increasing trend up to stage III with a peak of 892.47 pg/ml before decreasing in stage IV. Ovarian estradiol level gradually increased from stage I to III (maximum 1247.35 pg/g) and decreased in stage IV. On the contrary, progesterone level, though undetected in stage I, continuously increased up to stage IV in the haemolymph (876.23 pg/ml). However, in the ovary it gradually increased from stage I to IV (1368.44 pg/g). The levels of progesterone and estradiol were calculated with the help of one-way analysis of variance and their significance was expressed at less than 0.001. This study clearly indicated the positive correlation of progesterone quantity with the stages of ovarian maturation, but the estradiol level was negatively correlated.

Keywords : Vertebrate type steroids, ovarian maturation, mud crab.

Introduction

In crustaceans, the female reproduction is governed by a variety of hormonal and neuronal factors (Subramoniam, 2000). Gonad-inhibiting hormone (GIH) from the sinus gland and the gonad-stimulating hormone (GSH) in the brain and thoracic ganglion are the two antagonistic neurohormones contemplated to regulate gonad maturation in crustaceans. Crustacean hyperglycemic hormone (CHH) and androgenic hormone are also involved in reproductive control and methyl farsonate (MF) is thought to have a role as well (Adiyodi, 1985).

Hormonal control of vitellogenin synthesis and oocyte maturation are well documented in oviparous vertebrates (Tata and Smith, 1979; Wallace and Selman, 1981). The vertebrate steroids, like progesterone, estradiol, and testosterone are present in the ovary and haemolymph of crustaceans (Couch *et al.*, 1987; Van Beek and De Loof, 1988; Fairs *et al.*, 1989; Quinitio *et al.*, 1991). There are suggestions that progesterone may be a precursor in invertebrates, just as it is in vertebrates and has been reported to stimulate ovarian development and induce spawning in shrimps and prawns (Couch *et al.*, 1987; Quinitio *et al.*, 1991). Haemolymph progesterone and17β- estradiol increase at the onset of vitellogenesis and decrease after spawning in shrimps and prawns (Quinitio *et al.*, 1991).

The presence of vertebrate-type hormones, including progesterone, and 17β-estradiol is well documented in crustaceans but little is known of their role. Progesterone and 17β- estradiol have been found in the mandibular organ, kidney, hepatopancreas, ovary and haemolymph of *Scylla serrata* and levels of these hormones vary over the course of the reproductive cycle.

In the present investigation, an attempt has been made to study the changes in the levels of estradiol-17β and progesterone in the haemolymph and ovary of the mud crab *S.serrata* in relation to different stages of ovarian maturation as the information on the changes in these vertebrate-type hormones in relation to ovarian recrudescence is lacking.

Materials and methods

Female mud crab *S. serrata*, weighing 250 ± 10g were collected for a period of two months from Kovalam coast near Chennai and brought to the laboratory at Neelankarai where they were acclimatized to laboratory

conditions. During the period of acclimatization they were maintained in filtered sea water of 35 ppt salinity, 8.1 *p*H, 4.6 ml/l dissolved oxygen, 24-27 °C temperature, 1.15 mg/l ammonia, 1.36 μg/l nitrate and 1.36 μg/l nitrite. Then the animals were anaesthetized using MS 222 (100 μg/l) for a period of 10-15 minutes. First, the haemolymph was withdrawn from the base of chelate leg using a disposable syringe using sodium citrate (4:1 v/v) as an anti-coagulant. Next, the maturity stage of the animal was identified by examining the pleopod under the microscope. Later, the animals were sacrificed and the ovaries were dissected out. A part of the ovary was fixed in Bouin's solution for histological invèstigation and another part was stored at -70°C for radioimmuno assay.

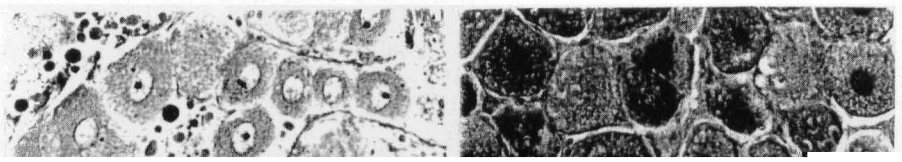
For histological investigation the tissue fixed in aqueous Bouin's fixative was dehydrated in alcohol, embedded in paraffin wax and was sectioned at 6μm. The sections were stained with haematoxylin and eosin (Bullock *et al.*, 1976). The changes during the ovarian development were observed under Zeiss microscope (Axioskop 2) and pictures were taken using an automatic exposure system. The ovary and haemolymph were extracted and proceeding to radioimmuno assay using scintillation counter. From this, the levels of 17β- estradiol / progesterone were quantified (Quinitio *et al.*, 1991). The levels of 17β-estradiol and progesterone in the haemolymph and ovary during the different stages of ovarian maturation were calculated with the help of one-way analysis of variance and the significance was expressed at less than 0.001.

Results

The ovarian stage I showed the presence of numerous oogonial cells with clear cytoplasm and prominent nuclei and nucleoli (Fig. 1.1). The clear cytoplasm found in the first stage got filled with small yolk granules during stage II. Due to this, the nucleus was not prominent as that was in the first stage. The size of the oocytes slightly increased (Fig. 1.2). In the third stage, as the cytoplasm got filled with yolk droplets the nucleus was not visible clearly. The size of the oocytes further increased (Fig. 1.3). The yolk droplets in the ooplasm become much larger to the extent that the oocytes appear to be large and matured occupying the entire ooplasm. Nuclei were not visible totally during the last stage of the ovary (Fig. 1.4).

17β-estradiol: Change in the levels of 17β-estradiol (Fig. 2) in the haemolymph was noticed during different stages of ovarian maturation. In stage I haemolymph, 17β-estradiol was not detectable. In stage II, 227.45 pg/ml was detected, in stage III, it was high (892.47 pg/ml) and in the stage IV, it decreased to 248.38 pg/ml.

Change in the levels of 17β-estradiol in the ovary was noticed during different stages of ovarian maturation. A level of 180.35 pg/g 17β estradiol was detected in stage



I which doubled to 491.51 pg/g in stage II with further 3 fold increase of 1247.35 pg/g in stage III. However, the 17β estradiol level decreased (603.22 pg/g) in stage IV (Fig. 2)

Progesterone: Change in the levels of progesterone in the haemolymph was recorded during different stages of ovarian maturation. It is interesting again to note that progesterone was not detected in the stage I haemolymph. However 89.43 pg/ml was detected in stage II that increased (4 fold rise) to 315.33 pg/ml at stage III and further to 876.23 pg/ml at stage IV (Fig. 3).

Change in the levels of progesterone in the ovary was also observed during different stages of ovarian maturation. A level of 120.29 pg/g progesterone was detected in stage I ovary, which increased to 191.55 pg/g at stage II with further increase (3 fold rise) of 765.46 pg/g at stage III. A maximum level of progesterone, 1368.44 pg/g was detected during stage IV (Fig. 3).

Discussion

Donahue (1957), found the eggs of *Homarus americanus* displaying substantial amount of estrogenic activity. Lisk (1961) not only confirmed the estrogenic activity in the eggs of *H. americanus*, but also identified it to be 17β- estradiol. In addition to bioassay method, Junera *et al.* (1977) employed a more sensitive and specific technique namely radioimmunoassay (RIA) to detect 17β- estradiol and estrone in the amphipod, *Orchestia gamarella pallas*. Subsequent studies demonstrated the presence of these hormones in the ovary, hepatopancreas and in the haemolymph (Couch *et al.*, 1987; Quinitio *et al.*, 1991, 1994). Nikitina *et al.* (1977) detected progesterone as well as testosterone in the whole body extracts

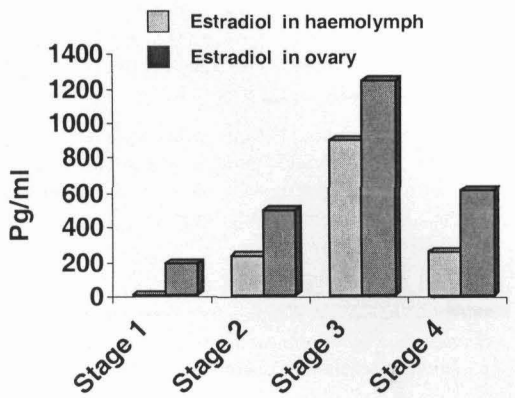


Fig. 2. Levels of Estradiol in haemolymph and ovary of *S. serrata* during ovarian maturation

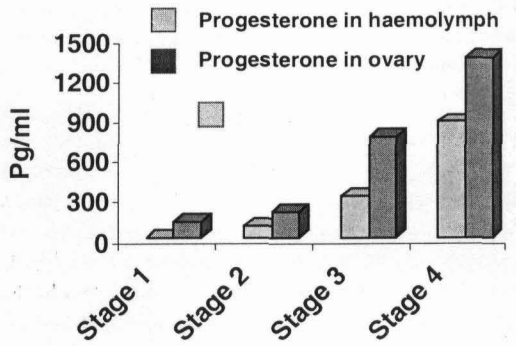


Fig. 3. Levels of Progesterone in haemolymph and ovary of *S. serrata* in ovarian maturation

of crustacean krill, *Euphausia superba*. Although the above authors established the existence of these vertebrate hormones in the invertebrate tissues, they made no attempt to study their relationship with egg development.

Estradiol and progesterone levels in the haemolymph are closely related to the stage of ovarian maturity. The levels of progesterone in the haemolymph were high in shrimps with mature ovary while those with immature ovaries were low or undetectable. Estradiol was detected only in stage V when the ovary was mature (Quinitio *et.al.*, 1994). Couch *et al.* (1987) observed high estradiol and progesterone levels in the serum of *H. americanus* with maturing ovaries. Ghosh and Ray (1993) have shown that the ovary and hepatopancreas are the sites of steroid metabolism in the prawn, *Macrobrachium rosenbergii*. Estradiol may stimulate the release of the molt-inhibiting hormone responsible indirectly for reproduction. Donahue (1955) suggested that ecdysis of berried *H. americanus* is normally inhibited by egg estrogen and perhaps also ovarian estrogen. High levels of estrogen during vitellogenesis may also suppress molting in *Penaeus monodon*. An untimely molt during vitellogenesis would result in a loss of sperm cells deposited in the thelycum on the cast exoskeleton.

In the present study, both ovary and haemolymph displayed increase in estrogen as well as progesterone. Estrogen was not detected in the first stage ovary. It was detectable in the second stage reaching a peak rise in stage III (4 fold increase- 892.47 pg/ml). But it decreased drastically in stage IV. On the contrary, progesterone level which was again not detected in stage I increased gradually up to stage IV reaching a peak level of 876.23 pg/ml. Ovarian estrogen level showed gradual rise from stage I to III with a peak of 1247.78 pg/g in III stage but decreased in IV stage. However, progesterone level in the

ovary followed their similar trend as haemolymph with continuous increase from I to IV stage reaching of peak of 1368.44 pg/g during the IV stage.

It was seen that maximum level of estrogen in the haemolymph when the synthesis of vitellogenin was at its peak in the ovary. Earlier workers have reported the ability of estrogen to stimulate the enzymes related to lipogenesis and ion transport in the hepatopancreas of the fresh water prawn *M. rosenbergii* (Ghosh and Ray, 1993). In vertebrates, progesterone is also a precursor to steroids including 17β- estradiol and other progesterone. In the ovaries of the crab, *Portunus trituberculatus,* progesterone yielded 17α-hydroxyprogesterone, testosterone and deoxycortisone (Teshima and Kanazawa, 1971). It is, therefore, possible that progesterone is precursor of testosterone synthesis which, in turn, is aromatized to form estradiol necessary for vitellogenesis in invertebrates such as *P. monodon.*

In most adult shrimps, the premoult stage is the longest during the molting cycle (Smith and Dall, 1985; Diaz and Petriella, 1990). In *P. monodon,* titers of conjugated 17β- estradiol and unconjugated and conjugated estrone in the ovaries peaked during vitellogenesis (Fairs *et al.*, 1990). These observations and the fact that exogenous steroids can stimulate ovarian maturation and spawning may indicate that 17β- estradiol plays a part in the reproductive process.

The present study clearly indicates that there is a gradual increase and decrease of the steroidal hormones17β- estradiol and progesterone levels both in the haemolymph and ovaries of *S. serrata.* Thus it can be confirmed from the present work that these hormones have definitely functional roles to play during the different stages of ovarian maturation and vitellogenesis.

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