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Profile of progesterone and estradiol in hepatopancreas, ovary, and hemolymph of shrimp *Penaeus chinensis* during reproduction cycle

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Abstract: The concentrations of progesterone and estradiol in hepatopancreas, ovary, hemolymph of *Penaeus chinensis* were investigated by means of radioimmunoassay during a reproductive cycle, and changes in GSI (gonadosomatic index) and HSI(hepatosomatic index) were monitored. Progesterone and estradiol were detected in the hepatopancreas, ovary, and hemolymph of the shrimp with maturing or matured ovary. During immature stage (length< 8- 10cm), the two hormones were undetectable, however, during previtellogenesis stage (perinucleolus stage, length> 11- 13cm), the concentrations of both hormones in three tissues increased rapidly, and estradiol in ovary reached a peak (450±86.7pg/g). During vitellogenesis stage (length> 13- 15cm), the two hormones maintained relatively high levels. Progesterone reached peaks in ovary (1975.1±175.2pg/g), and hepatopancreas (902.6±130.5 pg/g), and estradiol reached a peak in hemolymph (451.3 ± 73.7 pg/ml). The hormone level decreased sharply during maturation stage, and in hepatopancreas, the hormones even became undetectable. During whole reproductive cycle, GSI increased continuously and HSI stopped increasing during maturation stage, and showed a similar change like that of hormone levels in hepatopancreas and ovary. The results suggested that hepatopancreas might be the site of vitellogenin synthesis in *P. chinensis*.

Key words: *Penaeus chinensis;* progesterone; estradiol; reproduction cycle

1 Introduction

With the development of crustacean culture worldwide, the need for large quantities of high quality seed has become increasingly urgent. In order to meet the demand, endocrinological studies on cultured crustaceans such as *Penaeus chinensis*, *P. japonicus*, *P. vannamei*, *Eriocheir sinensis*, *Macrobrachium rosenbergii* etc. have been developed rapidly since 1980's (Jeng et al., 1978; Yano, 1985, 1987; Quackenbush, 1989; Ruo et al., 1990; Jiang et al., 1992; Zhao et al., 1992, 1996, 1999; Cai & Yang et al., 1998). Many researchers concentrated their studies on the relationship between steroid hormone and crustacean reproduction. Vertebrate-like hormones have been detected in various species of marine invertebrates (Sandor 1980, De Clerck et al., 1983; Vooget et al., 1984; Ollevier 1986). Couch et al. (1987) detected progesterone and estradiol in mandibular organ, hepatopancreas, ovary, and serum of the lobster *Homarus americanus* with developing and immature ovary. The

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two steroid hormones were also identified in the hemolymph of female *Pandulus kessleri* during the vitellogenesis stage and in hepatopancreas, ovary, and hemolymph of *P. monodon* during maturing stages (Quinitio et al. 1991, 1994). Zhao et al. (1999) demonstrated the existence of progesterone in the ovary of crayfish *Procambarus clarkii*, but none of the hormone was detected in the mandibular organ in her study. In this paper the changes of progesterone and estradiol level in three tissues (ovary, hepatopancreas, hemolymph), and compared this with the gonado-somatic index (GSI), and hepatosomatic index (HSI) of shrimp *P. chinensis* during the reproductive cycle were investigated. The relationships between shrimp production and the two steroid hormones are discussed.

2 Materials and Methods

2.1 Sample

Samples of *P. chinensis* were purchased from Liuting Shrimp Hatchery in Qingdao at four different times. The sample numbers, purchasing times, shrimp sizes, and ovary development states are listed in Tab. 1.

Tab. 1 shrimp sample in different of ages of ovary development(1)

stage	immature	pre-vitellogenesis	vitellogenesis	maturation
collecting time	1998/6/20	1998/9/20	1999/1/7	1999/3/20
Number of samples	10	10	10	10
Mean size(2) of samples (cm)	8.8±1.7	12.1±1.2	14.3±1.5	14.2±1.5
Size range of samples (cm)	8–10	11–13	13–15	13–15
Mating	no	no	yes	yes
Ovary development situation	no ovary outline observed from outside, the ovary slim, transparent, difficult to be separated, diameter of oogonia about 10 μm	ovary outline not clear observed from outside, dissected ovary half transparent, mean diameter of oocytes 40–80 μm, more than 8 circular nucleoli situated peripherally	greenish ovary can be observed outside, dissected ovary turnbig and plaque, mean diameter of oocytes 80–220 μm, oocyte was closed by a layer of follicle cell	ovary large and full, deep green or brown, mean diameter of oocytes 220–250 μm, yolk granule can be seen clearly, follicle cell become membrane-like material

(1): Shrimp grown from larva reared from April, 1st to April 20th in 1998.

(2): Body length is from the eyestalk base to the end of tail.

2.2 GSI and HSI

The ovaries and hepatopancreas were dissected out from the shrimp at different of ovary development stages. The gonadosomatic index (GSI) and hepatosomatic index (HSI) were calculated as follows:

GSI = gonad weight × 100/ body weight

HSI = hepatopancreas weight × 100/ body weight

2.3 Radioimmunoassay

2.3.1 Extraction

At first, a 0.2 ml hemolymph sample was drawn from the dorsal cardiac site with a syringe and placed in 1.5 ml plastic centrifugal tube with 1 ml ethyl acetate, as soon as it was bled from shrimp. The sample was centrifuged and extracted for two times mixed with ethyl acetate, and then the two extracts were combined. Next, 0.2g ovary and hepatopancreas tissue were dissected out respectively. Each sample was separately placed into a ground-glass tissue homogenizer to be homogenized, and was then extracted two times with ethyl acetate, then combining the extracts together. The extracts of three tissues were evaporated to dryness in a water bath (50–

55℃, and after the ethyl acetate was removed, the residue was resuspended by adding 1 ml 5% GPBS (gelatin phosphate buffer saline), and then kept at -20℃ for use.

2.3.2 Steroid measurement

The radioimmunoassay kits for detection of progesterone and estradiol were provided by Shanghai Biochemical Institute, Chinese Academy of Sciences, and the steroid measurement was conducted as the instructions given in the kits. A series of test tubes were prepared, which included sample tubes, standard tubes and control tubes. 0.5 ml GPBS-sample, a series standard of steroid hormones and control materials (reagent control, antibody control, and bland control) were added to the tubes, and then antibody and isotope were added. The tubes were put into the refrigerator (4 ℃) overnight. On the next day 0.2 ml of a dextran-charcoal solution was added to each tube. The solutions in the tubes were stirred and the tubes were placed in ice water for 20 min. After that, the tubes were centrifuged for 10 min. at 3000 rpm, and the supernatants were removed to liquid scintillation bottles, to which were added by 8 ml toluene PPO-POPOP mixture each tube. The radiation in the tubes was then read in a LKB 1209 Back-Beta liquid scintillation spectrometer.

2.3.3 Correction of extraction rate

100μL ^{3}H-progesterone (total CPM 2000) or ^{3}H-estradiol (total CPM 16000) was added into the 0.2g ovary, 0.2g hepatopancreas, and 0.2ml hemolymph separately, then mixed and set for 30 min. After that the samples were extracted with same method mentioned in 2.3.1. The dried extraction samples were solved in the 1ml 5% GPBS solution. Taking 0.5ml extraction solution from each sample and removing into the liquid scintillation bottles, to which were added by 8ml toluene PPO-POPOP mixture each bottle. The radiation in the bottles was then read in a LKP 1209 Bach-Beta liquid scintillation spectrometer. The extraction rate can be calculated through following formula.

Extraction rate (%) = CPM in extraction sample × 2 × 100/ total CPM

In this experiment, the mean extraction rates of progesterone in ovary, hepatopancreas, and hemolymph were 91.2±3.4%, 95.4±2.7%, and 87.7±5.2%, and the extraction rates of estradiol were 89.4±4.4%, 91.0±3.5%, and 88.9±2.9% (n=10), respectively.

2.4 Data analysis

The variance of GSI, HSI, the concentration of progesterone and estradiol during reproduction cycle was compared with one-way ANOVA, then the significant difference between groups was analyzed with *q*-test.

3 Result

3.1 GSI and HSI

The results of GSI and HSI showed an increase during reproductive cycle in *P. chinensis* (Tab. 2). GSI increased rapidly and continuously from the immature stage to the maturation stage. In each stage the value of GSI increased by 100% that of the preceding stage. Before vitellogenesis, the increase of GSI mainly reflected the active propagation of oogonia or oocytes. After that the increase showed the accumulation of yolk protein. HSI increased more slowly until vitellogenesis when the increase slowed or nearly stopped. The results suggested that before ovary maturation, hepatopancreas accumulated a great quantity of nutritional materials, which were then transported vie hemolymph for synthesis of vitillogenin, or was synthesized into vitellogenin in the hepatopancreas directly and then transported into the ovary where it accumulated. Close to maturation, when yolk protein synthesis had been accomplished, The HSI no longer increased.

Tab. 2 Variation of GSI and HSI in *P. chinensis* during reproductive cycle

ovary developing stage	immature	Pre-vitellogenesis	vitellogenesis	maturation
Age of sample(monthes)	2	5	8.5	11
GSI (n= 10)	1.1 ± 0.3$^{(a)}$	2.3 ± 0.8$^{(a)}$	4.6 ± 0.7$^{(b)}$	9.8 ± 2.6$^{(c)*}$
HSI (n= 10)	3.4 ± 0.4$^{(a)}$	4.9 ± 0.7$^{(b)}$	6.3 ± 1.0$^{(c)}$	6.7 ± 1.2$^{(c)}$

*: Different letters in same line show significant difference. (F_{GSI}= 75.39 F_{HSI}= 28.99 P< 0.01)

3.2 Progesterone

In the immature stage, progesterone has not been detected for any tissues (Fig. 1). However, during pre-vitellogenesis stage progesterone have been detected in three tissues, and the concentrations of the hormone were relatively high. Vitellogenesis stage, an active stage for ovary development, the hormone concentrations reached peaks in hepatopancreas (902.6 ± 130.5 pg/g) and ovary (1975.1 ± 175.2 pg/g). After that progesterone concentration decreased sharply, especially in hepatopancreas (no detection) and ovary (dropped by 70%). The hormone concentration was highest in the hemolyph (994.2 ± 102.5 pg/ml) during maturation stage.

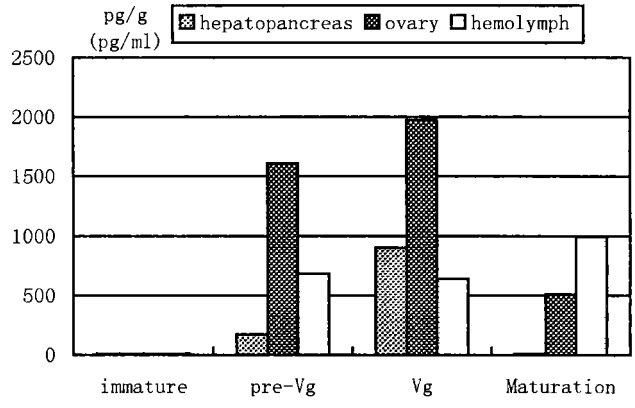

Fig. 1 Concentration of progesterone in three tissues of *P. chinensis* during reproductive cycle

3.3 Estradiol

Like progesterone, estradiol was not detected during immature stage (Fig. 2). Estradiol concentrations increased rapidly and reached peaks in hepatopancreas (222.6 ± 46.1 pg/g), and ovary (450.7 ± 86.8 pg/g) during previtellogenesis stage. From previtellongenesis to vitellogenesis stage (or during maturing stage) estradiol maintained at relatively high levels in three tissues (especially in hemolymph 451.7 ± 73.4). However when shrimp was close to mature, estradiol concentration dropped dramatically, especially in hepatopancreas where no hormone could be detected. Estradiol had a relatively high concentration in hemolymph during whole ovary developing stage. In all three tissues during different stages, estradiol concentration was about 1/5 of that of progesterone.

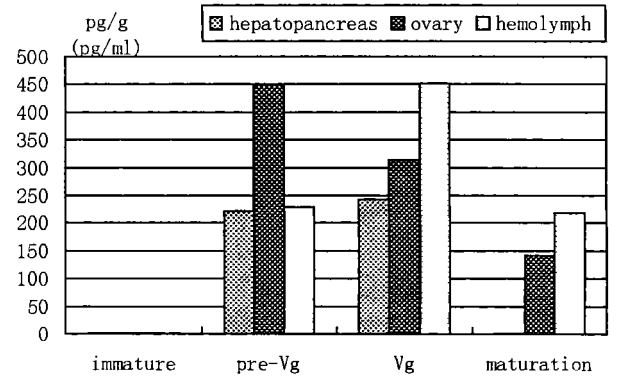

Fig. 2 Concentration of estradiol in three tissues of *P. chinensis* during reproductive cycle

4 Discussion

This is the first time to show the evidence of progesterone and estradiol existing in the tissues (hepatopancreas, hemolymph, and ovary) of the shrimp *P. chinensis*. The concentration of both hormones varied with the stage of ovary development.

Pre-vitellogenesis stage ovaries in adult shrimp showed increasing of both hormones. This is the stage of onset of ovary development, when the oogonium and oocyte propagate actively, and the nucleolus within the oocyte is situated peripherally. In the cytoplasm, various cell structures such as endo-plasmic reticulum, golgiosome, mitochondria etc. increased rapidly to prepare for yolk protein synthesis in next stage (Hong et al., 1998; Li & Zhang, 1994). The variation of progesterone and estradiol concentration was related to the vitellongenesis. The maturing stage (from pre-vitellogenesis to vitellogenesis stage) was characterized by the maintenance of high progesterone and estradiol levels. So the hormones may trigger ovary development and regulate yolk protein synthesis. When the ovary was close to mature, the hormone level dropped dramatically, especially in the hepatopancreas (undetectable) and ovary (3– 4 times lower).

Couch et al. (1987) reported that during the immature stage in *Hormarus americanus*, progesterone could be detected only in mandibular organ (MO), and that the estradiol level was very low or undetectable in the tissues including MO, hepatopancreas, blood, ovary etc. However, in the lobster with developing ovaries, the two hormones could be detected in all four tissues. Progesterone concentration was high especially in ovary and hepatopancres (1000– 1300 pg/g), but was similar to levels recorded in the immature stage for MO. He suggested that progesterone might be synthesized in the MO and secreted into blood where it was transformed into estradiol, and that it was estradiol that played an important role in crustacean ovary development.

Quinitio et al. (1991) checked the progesterone and estradiol levels in the serum of the shrimp *Pandalus kessleri* in an annual reproductive cycle. She found that progesterone level was highest during pre-vitellogenesis (286.2 pg/ml), and decreased during vitellogenesis stage. In contrast, the estradiol level was lower (18.1pg/ml) during the pre-vitellogenesis stage, and higher (54.3 pg/ml) during the peak of vitellogenesis. Except for the reproductive season, the two hormone levels were very low, or undetectable. Quinitio et al. (1994) also reported the variation of progesterone and estradiol in the hepatopancreas, hemolymph, and ovary of *P. monodon* during different reproductive stages. The results showed that during the immature stage, the two hormone levels were low or undetectable, but during the maturing stage the levels were higher. Obviously the hormones were related to shrimp ovary development.

In this research the results were similar to those of the above authors. The two hormone levels were very low or undetectable in the immature shrimp *P. chinensis*, but the concentration rose during reproductive stage, especially from pre-vitellogenesis to vitellogensis stage. The results further demonstrated that steroid hormones had a close relation to shrimp reproduction.

That progesterone or 17α-hydroxyprogesterone could induce shrimp ovary development has been reported by many authors. Yano (1985, 1987) injected *P. japonicus* with 17α-hydroxy-progesterone and found that 48 h later the concentration of vitellogenin in the serum increased three times. When he injected shrimp *Metapenaeus ensis* with progesterone, he found that the ovary development in treated shrimp was much quicker than that in control shrimp. Tsukimura & Kamemoto (1991) cultured the oocytes of *P. vannamei* in vitro and added 17α-hydroxy-progesterone to the culture media. The result showed that the hormone could stimulate a significant increase in the diameter of oocytes. Similar results were got by Zhao et al (1996) in *Macrobrachium rosenbergii* and by Cai & Yang (1998) in *P. chinensis*. In Cai' s trials the results showed that the mean oocyte diameter could increase by 30– 50% when stimulated by 2×10^{-6}– 10^{-8} g/ml of 17α-hydroxy-progesterone added to the culture medium.

The above results have showed that some steroid hormones like progesterone do affect shrimp reproduction, but little is known about the mechanism. In the vertebrate, progesterone is the precursor of other steroid hormones including estradiol, which can induce vitellogenesis of amphibian and fish (Redshaw 1972, Wang et al. 1990). Teshima & Kanazawa (1971) reported that the crab *Portunus trituberculatus* could transform progesterone into 17α-

hydroxy-progesterone, testerone and deoxycorticosterone etc. So Couch et al. (1987) suggested that, in crustacean, progesterone might be the precursor of estradiol.

Hepatopancreas is a site of vitellogenin synthesis in many kinds of crustacean (Paulus & Laufer 1987; Quackenbush 1989). From the immature stage to the vitellogenesis stage, HSI increased continuously, but almost stopped when *P. chinensis* was close to mature. The progesterone and estradiol levels were also high in the hepatopancreas from pre-vitellogenesis to vitellogenesis stage, but were undetectable in the mature stage. This result suggests that the hepatopancreas might be the site of vitellogenesis in *P. chinensis*, and that the hormones played an important role in vitellogenin synthesis.

Though have been demonstrated the existence of progesterone and estradiol in hepatopancreas, hemolymph, and ovary, little about the exact site of vitellogenin synthesis, the course of their metabolism and effect mechanism are known and they are worth further study.

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中国对虾肝胰腺、卵巢及血淋巴中的孕酮和雌二醇含量的生殖周期变化

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摘要: 首次证实了孕酮和雌二醇这两种类固醇激素在中国对虾体内的存在。在性腺未发育阶段, 肝胰腺、卵巢和血液中两种激素的含量均很低, 难以检测到。而在卵黄发生前期(核仁周边期), 三种组织中孕酮和雌二醇含量迅速上升, 卵巢的雌二醇含量达到高峰(450.1±86.7)。进入初级卵黄发生阶段, 三种组织中, 两种激素均具较高含量, 卵巢和肝胰腺的孕酮含量(分别为1975.1±175.2和902.6±130.5pg/g)以及血淋巴中的雌二醇含量(451.3±73.7)达到高峰。到了次级卵黄发生阶段, 孕酮和雌二醇的含量迅速下降, 肝胰腺等组织中几乎检测不到。对虾性腺指数(GSI)的增长既显著且有规律性, 每一期增长幅度都达到或超过100%。肝胰腺指数(HSI)从性腺未发育期(3.4±0.4)到卵黄发生前期(4.9±0.7)以及从卵黄发生前期到初级卵黄发生期(6.3±1.0)有显著的增长, 而从初级卵黄发生期到次级卵黄发生期HSI增长不显著(6.7±1.2)。肝胰腺指数的增长与两种类固醇激素含量的变化具相似的趋势。上述结果显示, 孕酮和雌二醇可能具有刺激和调控中国对虾性腺发育的作用, 肝胰腺可能是卵黄蛋白原的合成场所。

关键词: 中国对虾; 孕酮; 雌二醇; 生殖周期
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