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Endocrine regulation of vitellogenesis in lobsters

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Abstract

Endocrine control of reproduction in lobsters is reviewed**.** As in other malacostracans**,** female reproduction in lobsters is under a bi-hormonal regulatory system consisting of an inhibitory neuropeptide originating from the X-organ sinus gland complex resident in the eyestalk, and a variety of stimulatory hormonal factors of diverse chemical nature. Interestingly, in *Homarus americanus,* crustacean hyperglycemic hormones also play a part in stimulating vitellogenesis. While methyl farnesoate, a sesquiterpenoid secreted by mandibular organs as well as the molting hormone ecdysteroids have been implicated with a positive control of vitellogenesis, our recent investigations have uncovered a role of vertebrate steroids such as estradiol 17β and progesterone in the control of vitellogenesis and post vitellogenic meiotic maturation in the spiny lobster *Panulirus homarus*. In addition, biogenic amines such as 5-hydroxytryptamine stimulate the release of gonad stimulating neuropeptides from brain and thoracic ganglia of *P. homaraus*.

Keywords: Vitellogenesis, lobsters, endocrine regulation, lobster reproduction

Introduction

Successful culture of any crustacean requires a sound knowledge on its general biology as well as the mechanism and control of growth and gamete maturation. Growth in crustaceans is controlled by periodical molting but, unlike insects, continues beyond sexual maturity and gonadal function. The consequential alternation of molting and reproductive cycles in the malacostracans underlines an efficient endocrine regulatory mechanism to control these two highly energy-demanding processes in the adult crustaceans. This is particularly important for the large-bodied and hard-shelled decapods like lobsters, where the cost of exoskeletal formation and egg production is enormous. In the spiny lobster *Panulirus homarus,* the cast away cuticle after ecdysis could weigh as much as 25% of the total wet weight of the molted lobster, implying significant quantities of organic and inorganic substrates that go into the synthesis of cuticle at each molt. Similarly, the female lobster spends almost equal quantity of organic substrates for egg production. This entails highly regulated derivation of organic molecules from storage organs such as hepatopancreas for the formation of exoskeleton as well as enormous amount of yolk protein synthesis involved in egg production. Crustaceans seem to have achieved this temporal utilization of organic substrates both in cuticle formation and egg production by employing two sets of antagonistic endocrine factors, one inhibitory and other stimulatory, to establish a delicate balance between the controlling of these two physiological events. This review is a critical appraisal of the interactive roles played by various hormonal factors in coordinating molting and female reproduction in lobsters.

Vitellogenesis in lobsters

Lobsters are one of the decapod crustaceans that have received early attention on the characterization and formation of yolk materials during vitellogenesis. As early as 1937, Stern and Salomon described the major yolk protein to be "ovoverdin" by virtue of its green colour in the American lobster *Homarus americanus*. Subsequent studies revealed its immunological identity with a corresponding

hemolymph protein, namely vitellogenin (Byard and Aiken, 1984). This is in contradiction with an early electron microscopic work of Kessels (1968), who based on electron microscopic investigations, in several decapod crustaceans including lobsters, *Homarus* and *Panulirus* suggested that an autosynthetic mode of yolk formation. However, later ultrastructural studies indicated the derivation of yolk precursor materials from the hemolymph for pinocytotic uptake into the maturing oocytes of *H. americanus* (Schade and Shivers, 1980). These authors not only studied the surface specialization of the oocyte periphery associated with pincytotic activity during intense vitellogensis, but also demonstrated the penetration of peroxidase into the oocyte, employing a horseradish peroxidase tracer technique. Such a process of yolk protein uptake into the oocytes of *H. americanus* has also been observed during an artificially induced vitellogenesis by way of eyestalk ablation. Immunological relatedness between the egg protein, vitellin and its precursor, vitellogenin in the hemolymph has been indicated in subsequent studies substantiating the extra oocytic derivation of yolk precursor molecules in lobsters (Tsukimura *et al*., 2002). That the onset of vitellogenesis corresponded with the transition from short day length to long day length has also been indicated by the appearance and increasing concentration of vitellogenin in the hemolymph of *H. americanus*, cultured under photoperiod-induced artificial laboratory conditions (Nelson *et al*., 1998).

In lobsters, vitellogenesis is a dynamic process since many species produce large numbers of yolk-laden eggs, which are incubated externally for extended periods in the brood. It is now well established from recent molecular studies that hepatopancreas synthesizes most of the yolk proteins. Amino acid sequencing of lobster vitellogenin as well as the construction of cDNA has enabled detection of vitellogenin gene and its expression pattern in hepatopancreas and ovary. In the spiny lobster *H. americanus,* the vitellogenin gene (*HaVg1*) transcript levels are low during previtellogenesis, but increased to a maximum in the hepatopancreas during intense vitellogenesis (Tiu *et al*., 2009). The ovary also expressed a very low level of *HaVg1*. In this respect, lobsters are similar to those of the other large sized decapods such as crabs (Zmora *et al*., 2007).

Endocrine control of vitellogenesis in lobsters

Vitellogenesis in decapod crustaceans is under the control of a bi-hormonal mechanism consisting of vitellogenesis inhibiting hormone from the eyestalk and a variety of stimulatory hormones that differ among major crustacean groups. Different processes involved in both yolk precursor synthesis and its uptake into the mature oocytes and the subsequent meiotic maturation are under the interactive role of these inhibitory and stimulatory hormonal factors.

Vitellogenesis inhibiting hormone

Crustaceans are unique in possessing a set of neurohormones produced primarily in the eyestalk ganglia, involved in the control of several physiological functions. They are produced in the neurosecretory cells of the medulla terminalis X-organ of the eyestalk. Keller (1992) classified these neuropeptides to belong to CHH / MIH / GIH neuropeptides family. CHHs are the crustacean hyperglycemic hormones involved in the regulation of carbohydrate metabolism, while the GIH is important for the inhibition of vitellogenesis. MIH, the molt-inhibiting hormone prohibits molting. These rather large neuropeptides, consisting of 72-83 amino acid residues, show significant homology in their primary structure, often exhibiting cross functions, when experimentally tested. GIH plays a prominent role in the regulation of reproduction, e.g. inhibition of vitellogenesis in the females, and hence the name, VIH. GIH also controls male reproductive activities and gets expressed in juvenile lobsters (Wilder *et al.,* 2002).

Incidentally, lobster is the first crustacean group in which the GIH has been molecularly characterized and the primary structure of this neuropeptide delineated (Soyez *et al*., 1987). Lobster VIH is a 78-residue peptide with a molecular weight of 9135 Da and having an amidated C-terminus and free N-terminus in *H. americanus*. Nevertheless, VIH of this lobster is present in the sinus gland as two isoforms with identical amino acid sequences and molecular masses, but with a different elution pattern

in HPLC analysis, possibly due to a different folding of the peptide (Soyez *et al.,* 1994). Interestingly, only one of the isoforms is biologically active in inhibiting vitellogenesis. Although the mechanism of its action is not fully understood, GIH might directly or indirectly regulate the production and / or release of other hormones involved in reproduction. However, the available evidence indicates that VIH could inhibit the uptake of vitellogenin into the oocytes, probably interfering with vitellogenin binding to its membrane receptor in the lobster *H. americanus* (Soyez *et al*., 1991). Ohira *et al.* (2006) recently produced a recombinant peptide related to vitellogenesis-inhibiting hormone (VIH) of the American lobster *H. americanus*. They studied the biologically active principle in the peptide and found that the amidated C-terminus is responsible for the vitellogenesis inhibition. Unlike

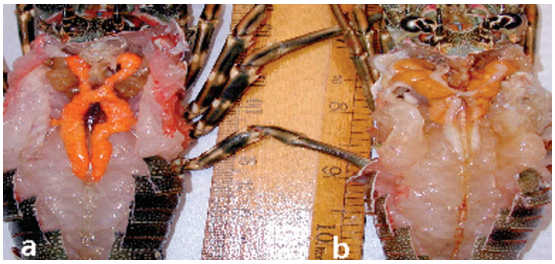

Fig. 1. a. Bilaterally ablated female lobster (below 100 g body wt.), note the fully developed H shaped ovary with orange colour. b. control-unablated female lobster of the same size

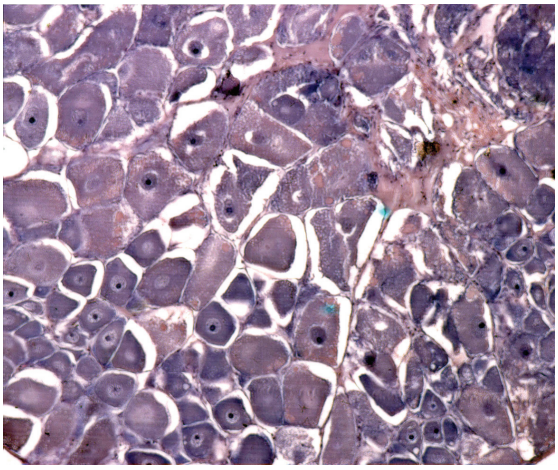

Fig. 2. C.S of the previtellogenic ovary of the unablated lobster (hematoxylin stained)

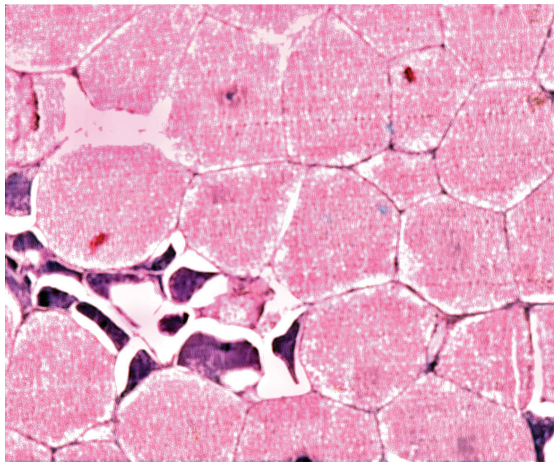

Fig. 3. Note the yolk laden fully mature oocyte (eosinophilic) in the bilaterally eye ablated lobster

the lobster MIH, the preproGIH has high degree of identity with crab preproMIH, suggesting that MIH and GIH form a group from other CHH neuropeptide family members. Removal of the eyestalk in the American lobster in the autumn accelerates ovarian maturation and spawning (Waddy *et al.,* 1995). In our own experimental study on the spiny lobster, *Panulirus homarus* (Linnaeus, 1758), both unilateral and bilateral eyestalk ablation induced ovarian maturation in immature lobsters weighing less than 100g body weight (Figs. 1- 3).

Gonad stimulating hormones

Unlike the gonad inhibitory factor in the form of neuropeptide, the gonad stimulatory principles in crustaceans are quite diverse. Early studies suggested the occurrence of a stimulatory neuropeptide (GSH or VSH) in the brain and thoracic ganglion of several decapod crustaceans to control vitellogenesis (Adiyodi and Subramoniam, 1983). Although the chemical nature of this putative vitellogenesis / gonad stimulatory neurohormone has not been fully characterized, several experimental studies have proved the existence of this stimulatory principle in crabs and shrimp (Subramoniam, 1999). Injection of brain extracts from *H. americanus* induced ovarian maturation in the shrimp, *Penaeus vannamei*, to suggest either a direct influence of lobster GSH on shrimp ovary or a stimulatory role to release the GSH from the brain and thoracic ganglia of the

shrimp (Yano and Wyban, 1992). Unfortunately, neither the molecular characterization nor the mechanism of their positive action on vitellogenesis has been investigated in lobsters or in other decapod crustaceans. Interestingly, CHHs of *H. americanus* has been shown to exhibit a positive control over vitellogenesis. HPLC fractionation of sinus gland of *H. americanus* yielded CHH-A and CHH-B isoforms (Tensen *et al*., 1989). Expression studies of both these CHH isoforms have revealed that these hormones are produced throughout the nervous system. More interestingly, the levels of CHH-B mRNA are low immediately after spawning and increased to significantly higher levels when vitellogenesis resumes after spawning. Hemolymph CHH-A and CHH-B levels are similarly higher just before spawning, suggesting that CHH may also be involved in oocyte maturation (de Kleijn, 1995). The balance of CHH and GIH expression may tune the synchronization of reproduction and growth during the reproductive cycle of *H. americanus*.

Role of biogenic amines: In this context, the role of different biogenic amines in influencing the release of peptides from different neurosecretory neurons is relevant to understand their integrative role in crustacean vitellogenesis. Biogenic amines such as dopamine and serotonin (5-HT) act as neuroregulators to control several physiological processes in crustaceans (Fingerman, 1997). HPLC-EC detection of 5-HT and its immnuocytochemical localization in the brain and thoracic ganglion of the Indian spiny lobster, *Panulirus homarus,* have revealed a rise in the synthesis of this biogenic amine in the nerve cells, correlated to ovarian

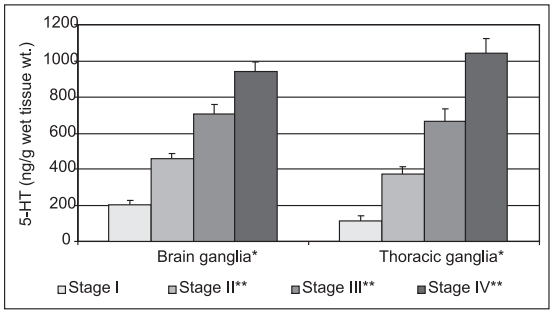

Fig. 4. Changes in the levels of 5-hydroxytryptamine in brain and thoracic ganglia during various stages of ovarian recrudescence in *Panulirus homarus*

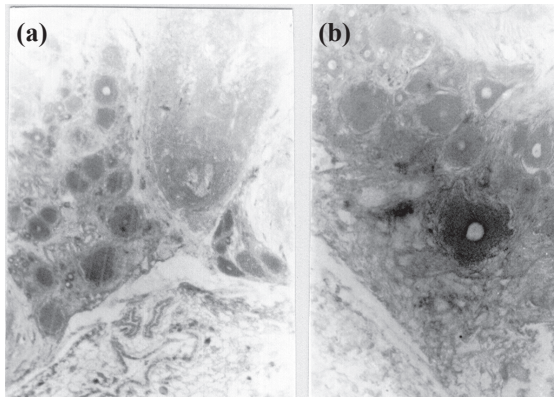

Fig. 5 a & b. CS of brain ganglia showing immunoreactivity for 5-HT during vitellogenic and post vitellogenic stages

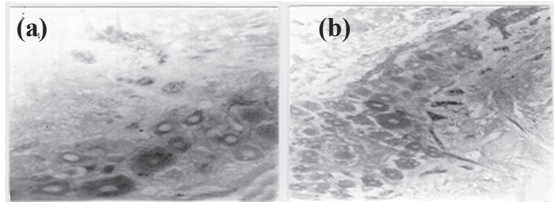

Fig. 6a & b. CS of thoracic ganglia showing immunoreactivity for 5-HT during vitellogenic and post vitellogenic stages

maturation (Fig. 4; Kirubagaran *et al*., 2005). Immuno-cytochemical studies have also indicated a corresponding increase in the expression of 5-HT in the brain and thoracic ganglia neurons during ovarian maturation (Figs. 5a & b and 6 a & b). Apparently, these biogenic amines have a role in stimulating the release of neurosecretory hormones involved in vitellogenesis, rather than having a direct influence on ovary or yolk precursor synthetic tissues (Sarojini *et al*., 1966).

Methyl farnesoate: *O*ther than the neurosecretory hormones described above, information on the gonad stimulatory hormones of lobsters centers on mainly methyl farnesoate and vertebrate steroids such as estradiol 17 β and progesterone. Mandibular organs of lobsters secrete methyle farnesoate (MF) and farnesoic acid (Byard and Aiken, 1984; Tobe *et al*., 1989). MF is the precursor of insect juvenile hormone (JH III), and hence it has been suggested to have a gonadotrophic function, just like insects. However, discordant results have been presented regarding

MF's role in reproduction. In crayfish and certain crabs, the results are indicative of a positive role in vitellogenesis, but its role in the lobster reproduction is rather uncertain. Female *H. americanus* in which mandibular organs are removed continued to spawn normally and that MF levels in the hemolymph are high in ovigerous females during the winter, when there is no ovarian activity and the serum vitellogenin levels are low. When vitellogenesis is induced in the spring, MF drops to undetectable levels (Tsukimura, 1992). Contrasting results have however been reported recently on the same lobster species (*H. americanus*), in which a positive correlation has been shown to vitellogenic cycle (Tiu *et al.,* 2006). Using *in vitro* explant culture, these authors showed that treatment of hepatopancreas fragments with farnesoic acid (and not MF), another intermediary in the synthetic pathway of insect JH III, resulted in a significant stimulation in lobster vitellogenin gene (*HaVg1*) expression. Interestingly, treatment of hepatopancreas of this lobster with 20-HE also raised the Vg transcript levels in the medium.

Vertebrate steroids: Evidently, hormonal regulation of lobster reproduction is still contentious. Nevertheless, a long-standing observation in crustaceans indicates the steroidogenic ability of crustacean tissues including testis and ovary in producing compounds such as pregnenolone, progesterone, and 17β-estradiol. In *H. americanus*, progesterone and 17β-estradiol have been found in the mandibular organ, kidney, ovary and hemolymph, with mandibular organ showing the highest concentration (Couch *et al*., 1987). There seems to be a correlation between the level of estradiol and ovarian development, as estradiol is found only in lobsters with maturing ovaries. Similarly, our studies on the spiny lobster, *Panulirus homarus*, also indicate a positive correlation between hemolymph 17β-estradiol and progesterone levels and ovarian cycle (Fig. 7; Kirubagaran *et al*., 2005). These two hormones were not detectable when the oocytes are in stage one immature stage, but increased in the hemolymph significantly during periods of vitellogenesis suggesting a role in the control of yolk synthesis and post meiotic oocyte maturation, as in oviparous vertebrates. We have similar results for a role of 17β-estradiol in stimulating vitellogenic activities in other decapods such as a mole crab, *Emerita asiatica* and the giant freshwater prawn *Macrobrachium rosenbergii* (Gunamalai *et al.,* 2006). In analogy with oviparous vertebrates, 17β-estradiol could transcriptionally activate vitellogenin gene in crustaceans too. A recent discovery of nuclear receptors for both progesterone and estrogen in a freshwater crayfish has lent support to the above conclusion (Paolucci *et al*., 2002). More molecular data is needed for further confirmation of this contention that vertebrate steroids control vitellogenesis in lobsters and other crustaceans in which correlative fluctuation of these steroids with ovarian cycle occurs.

In the aquatic vertebrates, the transcriptional activation of vitellogenin gene by estradiol 17 β has been used as a molecular marker to investigate levels of organic pollutants, which mimic estrogenic activity. When the fishes are exposed to such pollutants, the males have vitellogenins in the circulation, thereby indicating the presence of such estrogenic pollutants in the medium. Lobsters are marine benthic organisms and hence they are liable to be affected by the sedimental pollutants, which could contain xenoestrogenic compounds. Since the complete cDNA encoding vitellogenin for lobsters is now known (Tiu et *al*., 2009), molecular detection of mRNA expression for vitellogenin gene could be made in the hepatopancreas of the male lobsters living in polluted environment.

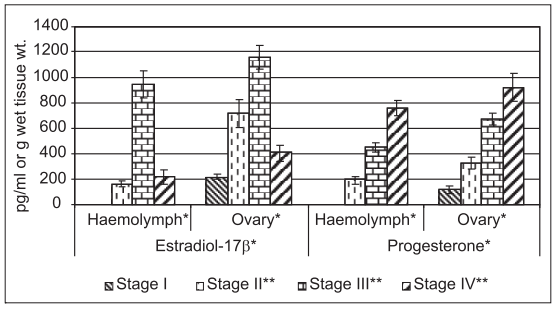

Fig. 7. Changes in the levels of estradiol-17β and progesterone in the hemolymph and ovary during various stages of ovarian maturation in *Panulirus homarus*

Conclusions

By far, a clear understanding on the mode of yolk formation has been reached on model decapod

crustaceans such as the crabs, shrimp, and lobsters from recent physiological and molecular studies. As regards the site of vitellogenin synthesis, majority of the crustaceans employ hepatopancreas as the main synthetic organ, although in the penaeid shrimp, the contribution from the ovary is equally significant (Wilder *et al*., 2002). The earlier electron microscopic observations implicating the oocyte of crayfish and crabs, as the site of yolk synthesis, have turned out to be proteins other than vitellogenin. However, the crustacean model for vitellogenin synthesis agrees with insects, in that the fat body is the primary site of vitellogenin synthesis in the majority of insects, with dipteran flies relying on ovarian follicle cells to synthesize the yolk peptides (Raikhel and Dhadilla, 1992). Receptor-mediated yolk protein uptake into the oocytes has also been demonstrated in several crustacean including lobsters. Despite these similarities in the mechanism of vitellogenesis, there is considerable variation existing in the hormonal control of crustaceans. Lobsters being the most conspicuous and commercially important crustaceans have attracted significant attention from endocrinologists on the control of both reproduction and molting. Briefly, lobsters are similar to other decapod crustaceans in their endocrine mechanisms to control these physiological processes, but the role of eyestalk neuropeptides in the regulation of vitellogenesis is highly complicated in lobsters. Thus, both viellogenesis inhibiting and –promoting factors exist in the form of VIH and CHHs. Among the many endocrine factors that exert a positive control over ovarian maturation, vertebrate steroids like estradiol and progesterone are shown to have dominating influence on vitellogenesis in lobsters. Our radioimmuno assay of these hormones during ovarian maturation in *P. homarus* has provided crucial clues for their transcriptional control of

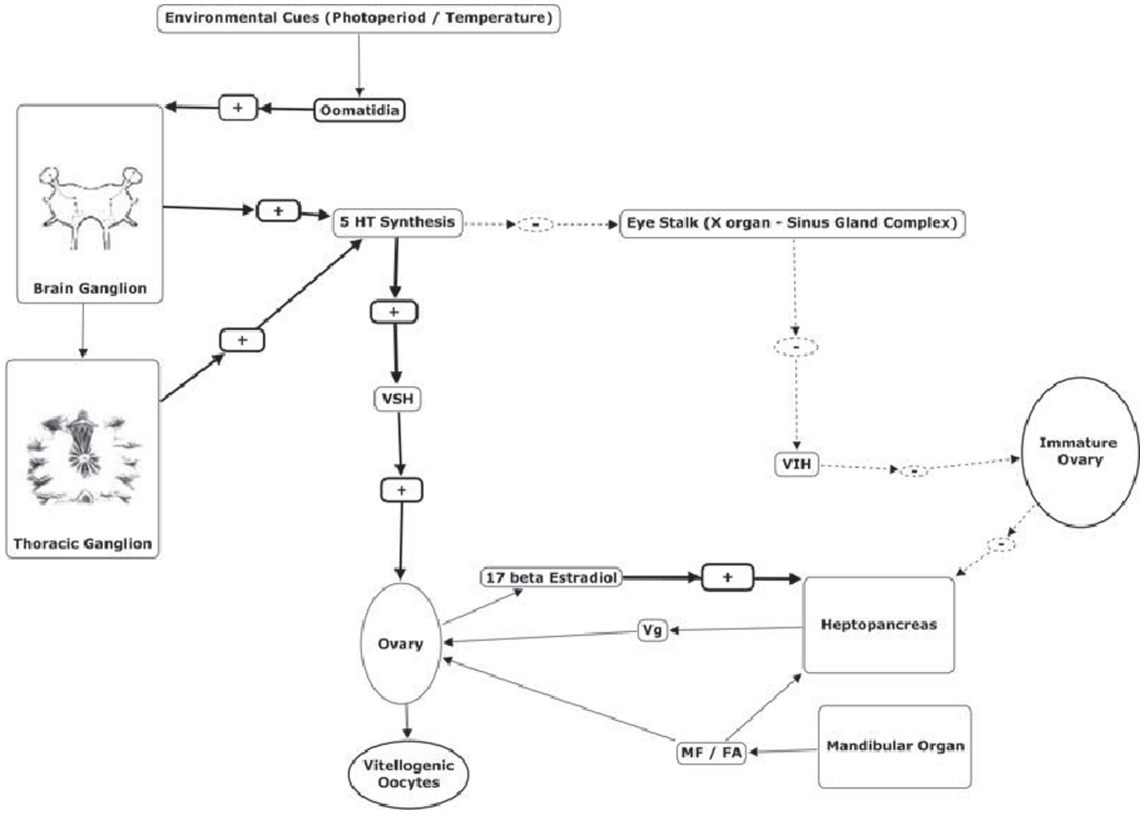

Fig. 8. Possible endocrine medchanism in lobsters; Vg-Vitellogenin, MF-Methyl Farnesoate FA- Farnesoic Acid, VIH – Vitellogenin Inhibitory Hormone

vitellogenin synthesis as well as post-vitellogenic meiotic maturation. In addition, biogenic amine-controlled gonad stimulatory neuropeptides from brain and thoracic ganglion could also exert direct and/or indirect influence revealing multi-hormonal control over vitellogenesis. Fig. 8 depicts the possible endocrine regulatory mechanism in lobsters to control vitellogensis. This complicated bi-hormonal regulation of vitellogenesis in conjunction with alternate control of molting process represents a highly evolved endocrine regulatory mechanism to control growth and reproduction in crustaceans. The existence of two steroid hormones such as ecdysteroids and vertebrate steroids to control molting and reproduction in crustaceans have important implications in their coordination of these vital processes to enhance both growth and reproduction by judicious channelization of energy-providing substrates from storage centers like hepatopancreas.

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