

*Review Article***Steroidal Control of Vitellogenesis in Crustacea: A New Understanding for Improving Shrimp Hatchery Production**

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Hormonal control of oocyte maturation in crustaceans is reviewed. The bihormonal control of egg maturation is accomplished by the inhibitory neuropeptides originating in the X-organ/sinus gland neurosecretory system and a host of stimulatory hormonal factors secreted from various sources. Among the gonad stimulatory hormones, steroids play a pivotal role in the control of molting and reproduction in crustaceans. Whereas ecdysteroids control molting, vertebrate sex steroids such as estrogen and progesterone seem to control vitellogenic activities. A protocol to stimulate vitellogenesis and egg maturation in the commercially important marine and fresh water shrimps by combining eyestalk ablation with sex steroid hormone treatment is proposed.

Keywords: Crustaceans; Sex steroids; Ecdysteroids; Eyestalk Ablation; Seed Production; Aquaculture

Introduction

Crustacean endocrinology is at the crossroads of its possible contribution to the development of shrimp aquaculture, which is hampered by lack of appropriate technology for seed production. A multitude of research publications on endocrine regulations of reproduction and molting have flooded the literature in recent times, owing to an increased attention focused on the aquaculture of species such as shrimp, lobster and crab. The worldwide-accepted protocol to induce maturation and spawning of penaeid shrimp species is eyestalk ablation, by which the source of gonad inhibitory hormone (GIH or VIH : gonad/vitellogenesis inhibiting hormone) i.e. X-organ/sinus gland complex is removed. Despite its easy maneuverability and precocious maturation and spawning of the wild-caught shrimps, the technique of eyestalk ablation has its inherent setbacks in producing quality seeds.

Nevertheless, recent studies have clearly indicated that VIH exerts its inhibitory influence on vitellogenin synthesis in the ovary and hepatopancreas of different decapod crustaceans (Wilder *et al.*, 2010). Most studies of VIH influence on vitellogenin

synthesis have been made under *in vitro* conditions, using ovarian explants of penaeid shrimp, indicating the negative effects of VIH on the ovarian synthesis of vitellogenin (Tsutsui *et al.*, 2005; 2007). However, other studies (Jayasankar *et al.*, 2002; Chen *et al.*, 2014) have revealed that hepatopancreas also responds to VIH in a similar way. Clearly, eyestalk extirpation alone is insufficient to induce yolk synthesis, and not adequate enough to yield healthy seeds, warranting alternative methods of hormonal intervention to achieve more success in seed production of commercially important shrimps. In this regard, recent studies have unraveled the existence of several hormonal factors that are shown to exhibit gonadotrophic effect on ovarian maturation and spawning (Subramoniam, 2016). Hence, more effective control of egg maturation under captive conditions could be achieved only by combining eyestalk ablation and administration of gonad stimulatory hormones. This review endeavors to analyze the existing information on the hormonal regulation of egg maturation in commercially important shrimp species to suggest ways to improve brood stock production and overall aquaculture productivity.

Hormones Controlling Ovarian Functions

It is well understood that malacostracan crustaceans employ a bihormonal regulatory system to control reproduction. GIH/VIH is the major eyestalk neuropeptide that inhibits the ovarian activities in Crustacea. A gonad stimulatory hormone (GSH), secreted from the brain and thoracic ganglia, was first proposed to act in antagonism with the GIH (Adiyodi and Adiyodi, 1970). This concept was in vogue, until the other gonad stimulatory hormones from non-neural sources were discovered. Furthermore, continued body growth in the sexually mature malacostracan species imposes additional stress on the temporal utilization of stored energy for molting and reproductive functions. In this regard, the discovery of yet another inhibitory hormone (Molt Inhibiting Hormone, MIH), originating from the X-Organ/ sinus gland complex, posits plausible interplay between GIH and MIH in coordinating the molting and reproductive processes (Adiyodi and Subramoniam, 1983).

In addition, crustacean hyperglycemic hormone (CHH) and MIH, with their known functions in glucose metabolism and molt inhibition, were shown to possess positive influence on vitellogenin synthesis in different decapods, including lobster, penaeid shrimp, and brachyuran crab (De Kleijn *et al.*, 1995; Gu *et al.*, 2002; Zmora *et al.*, 2009 a, b). Such cross functionality found among CHH family peptides is only expected, considering the structural homology existing among them (Keller, 1992; Webster *et al.*, 2013). Furthermore, recent reports have uncovered the occurrence of several vertebrate-type gonadotropic hormones such as Follicle-stimulating hormone (FSH)- like peptides in the brain and ovary, Gonadotropin-releasing hormone (GnRH) as well as bursicon in the brain and thoracic ganglia of shrimp (Sathapondecha *et al.*, 2015; Guan *et al.*, 2013). It is tempting to suggest that the decapod central nervous system is bipartite to produce inhibitory hormones in the optic ganglia, and the gonad stimulatory hormones in the brain and thoracic ganglia.

The other gonad stimulatory hormones thus far unraveled are: biogenic amines and opioid peptides, originating from the brain and thoracic ganglia; methyl farnesoate, secreted from the mandibular organ; ecdysteroids from Y-organ, and the vertebrate-type steroids such as 17 β -estradiol and progesterone from the ovary or hepatopancreas (Subramoniam, 2000).

Since the effects of these hormonal factors were described individually in different crustacean species, generalization of a gonadotropic role of any particular hormone for the entire crustacean taxa is untenable.

Influence of Biogenic Amines

Biogenic amines function as neurotransmitters and neuromodulators, involving themselves in the regulation of different physiological activities (Fingerman, 1997). Serotonin (5-hydroxytryptamine, 5HT), for example, acts as a neurotransmitter and neurohormone with gonadotropic influence in crustaceans (Richardson *et al.*, 1991; Vaca and Alfaro, 2000). In the spiny lobster, *Panulirus homarus*, 5-HT showed increased synthesis in the brain and thoracic ganglia, correlated to ovarian maturation (Subramoniam and Kirubakaran, 2010). The serotonin derivative such as melatonin is also shown to be involved in the integrative control of ovarian maturation in the freshwater crab, *Oziothelphusa senex senex* (Sainath and Reddy, 2011). On the contrary, dopamine plays an antagonistic role on the action of 5-HT in the stimulation of ovarian development in several decapods. Furthermore, Met enkephalin, an opioid, produced in the brain also possesses similar inhibitory effect on the oocyte maturation in the fresh water crayfish, *Procambarus clarkii* (Sarojini *et al.*, 1995). Serotonin-treated shrimps were reported to have advantage over eyestalk ablated shrimps with better egg quality, higher hatching rate, and higher production of nauplii (Meeratana *et al.*, 2006). In all these studies, the stimulatory action of serotonin is reported to be indirect, by stimulating the release of putative gonadotrophic substances from the brain and thoracic ganglion (Fingerman, 1997; Meeratana *et al.*, 2006; Sarojini *et al.*, 1995) or by inhibiting the gonad inhibiting hormone from optic lobes (Kulkarni *et al.*, 1992). However, in the tiger shrimp, *P. monodon*, Ongvarrasopone *et al.* (2006) have reported expression of a 5-HT receptor, 5-HT₁, in the membrane of mature oocyte in stages III and IV of the ovarian cycle. Our recent observations on the Indian white shrimp, *Fenneropenaeus indicus*, revealed differential expression of ovarian genes involved in vitellogenesis (Vg and VgR) in serotonin-treated female shrimps (Tomy *et al.*, 2016). Increase in the Vg and VgR gene expression was however higher in the eyestalk-ablated females, receiving serotonin treatment.

Evidently, 5-HT enhances release of gonad-stimulating brain neuropeptides in order that the latter can stimulate the proximate gonadotropic hormonal sources to initiate vitellogenesis, especially in the absence of VIH.

Effect of Methyl Farnesoate

Methyl farnesoate (MF), synthesized in mandibular organ, is the unepoxidated form of insect juvenile hormone, JH III (Borst *et al.*, 1987), and hence considered as a crustacean juvenoid. The evolution of MF in crustaceans in the place of JH in insect implied a gonadotropic role in crustaceans. Injection of MF or its precursor farnesoic acid stimulated vitellogenesis in a few crustaceans (Laufer *et al.*, 1986; Rodriguez *et al.*, 2002; Mak *et al.*, 2005; Tiu *et al.*, 2006). MF also binds to its receptor RXR in the ovary and hepatopancreas of the crab *Carcinus maenas* by forming a complex that could stimulate vitellogenesis (Nagaraju *et al.*, 2011). In *C. maenas*, RXR mRNA levels increased in the hepatopancreas and the ovary in a stage dependent manner during vitellogenesis. Expression of RXR in the vitellogenic ovary has also been reported in another brachyuran crab *Paratelphusa hydrodromous* (Sarika and Anilkumar, 2014). However, MF has a more prominent role in the control of male sexual behavior and in the sex determination of micro crustaceans such as *Daphnia* (LeBlanc, 2007). Previous work has also indicated that an interplay between ecdysone and methyl farnesoate could have a controlling effect on the allometric/ non-allometric growth in crustacean species in which several male morphotypes are present (Laufer *et al.*, 2002). In addition, MF exerts a positive control over ecdysone synthesis in the Y-organ (Tamone and Chang, 1993). The fact that MF synthesis is under the control of mandibular organ inhibitory hormone (MOIH) might suggest that this terpenoid compound plays a regulatory role in the coordination of reproduction and molting in decapods (Subramoniam, 2011).

Steroid Hormones

Steroids are widespread molecules derived from cholesterol and used by animals as hormones controlling reproduction, development and homeostasis (Lafont and Mathieu, 2007). In all oviparous vertebrates, sex steroids are the sole activators of vitellogenin (Vg) gene transcription (Wallace, 1985).

In invertebrates, a new class of polyhydroxylated ketosteroids, the ecdysteroids, native to arthropods, serve the primary function of molting hormone (Buchmann, 1989). The occurrence of the “native hormone” ecdysteroid and the “naturalized hormone” vertebrate – type sex steroids in a wide array of invertebrates ranging from the basal metazoans such as sponges and corals to advanced mollusks and echinoderms indicate their prominence in steroid evolution even before the origin of vertebrates. In the Pacific oyster *Crassostrea gigas*, the estrogen level in the ovary was correlated with Vg mRNA expression in the ovary (Matsumoto *et al.*, 1997, 2003). In oyster and scallop, estrogen treatment stimulated vitellogenesis, mediated by estrogen receptor (Osada *et al.*, 2003; Matsumoto *et al.*, 2007). In *C. gigas*, the cgER as well as the Vg mRNA were expressed only in the follicle cells, suggesting that estrogen receptor could regulate the transactivation of the Vg gene in the nuclei of the follicle cells (Matsumoto *et al.*, 2007). In addition, cgER expression was also seen in the nuclei of the oocytes, indicating that estrogen plays various roles in reproductive events in mollusks.

In other invertebrates such as the polychaete worm, *Neries virens*, injection of estradiol-17 β resulted in the synthesis of vitellogenin in the specialized coelomocytes, the eleocytes, in a sex-specific manner (Garcia-Alonso *et al.*, 2006). In addition, aromatase activity in the coral tissues as well as the occurrence of estradiol in coral eggs suggest a role for the latter in the control of egg maturation (Twan *et al.*, 2003). Furthermore, in the scleractinian coral, *Euphyllia ancora*, GnRH activity has been detected in the polyps during spawning season, indicating that GnRH has an ancestral role in the control of steroidogenesis and reproduction long before the appearance of the pituitary as a relay, with the emergence of the vertebrates (Twan *et al.*, 2006).

In Crustacea, steroids occur in two major forms, the ecdysteroids and the vertebrate-type sex steroids. Apart from their primary role in the regulation of molting, crustacean ecdysteroids also serve as gonadotrophic hormones, whereas vertebrate steroids have been implicated with a stimulatory role in vitellogenesis of several crustacean species (Subramoniam, 2016).

Influence of Ecdysteroids on Vitellogenesis

As in other arthropod groups, crustecdysone is the molting hormone in Crustacea. In contrast with insects, many crustaceans continue to molt in the reproductive adulthood, and hence in females, vitellogenic cycles are either synchronous or occur alternately with molt cycle (Subramoniam, 2000). Since the Y organ of crustaceans is active in synthesizing ecdysone in the reproducing females, the molting hormone could intervene with the control of vitellogenic activities. In a parallel situation found in insects, the hormone-secreting prothoracic gland atrophies in the imago stage, but the ecdysteroids are produced in an alternative source, ovary, to control various oogenic processes such as gonial mitoses, resumption of meiosis, vitellogenesis and ovarian cyclicity (Belles, 1998).

In crustaceans, the proposed function of ecdysteroids in female reproduction relied more on correlations (eg., between vitellogenesis and hemolymph ecdysteroid titer) than on direct experimental evidence. For example, Arvy *et al.* (1954) found evidence that there is a rise in hemolymph ecdysteroids coincident with the initial stages of oogenesis in the shore crab *Carcinus maenas*. It is also likely that ecdysteroids could be involved in the resumption of oocyte meiosis in the spider crab *Acanthonyx lunulatus* (Chaix and De Reggi, 1982) and in the palaemonid shrimp, *Palaemon serratus* (Lanot and Cledon, 1989). On the other hand, Steel and Vafopoulou (1998) found hemolymph Vg levels paralleling ecdysteroid titers during vitellogenic cycle of isopods and amphipods, suggesting a role in Vg synthesis. Again, in the amphipod, *Orchestia gammarellus*, a high titer of 20-hydroxyecdysterone (20E) appears to be necessary for the Vg synthesis in the fat body, as Y-organectomy in postecdysis inhibits the onset of vitellogenesis (Blanchet-Tournier, 1982). In a similar way, Okumura *et al.* (1992) found a close correlation between hemolymph ecdysteroid titer and ovarian maturation stages in the reproductive molt cycle of the freshwater prawn *Macrobrachium rosenbergii*. In the sand crab, *Emerita asiatica*, exhibiting synchronization of molting and oogenic cycles, hemolymph ecdysteroids revealed a biphasic increase with a small peak during intermolt, corresponding to vitellogenic activities in the ovary, and a prominent premolt peak (Gunamalai *et al.*, 2004).

In this crab, injection of 20E also enhanced protein synthesis in the ovary, hepatopancreas, and integumentary tissues, substantiating a dual role in the control of molting and reproduction. As suggested by the above authors, the effects of ecdysteroids on the control of vitellogenesis in Crustacea may be indirect; probably playing a metabolic role in the synthesis of different yolk precursor components both in the ovary and hepatopancreas.

Recent studies have furthermore revealed a positive correlation between Vg gene expression in the ovary and hepatopancreas and hemolymph ecdysteroid titers in the tiger shrimp, *Penaeus monodon* (Tiu *et al.*, 2006). Injection of ecdysteroids stimulated vitellogenesis in *P. monodon*, although the exact mechanism of its action is yet unclear. The mechanism of ecdysteroid action in the control of Vg in crustaceans could be resolved only by molecular studies pertaining to their receptor activities. In crustaceans, ecdysteroid receptor (EcR) has been identified in blastemal tissues of regenerating limbs of the crab *Uca pugilator*, and it heterodimerizes with retinoid X receptor, RXR, which is orthologous with ultraspiracle (USP) in insects (Chung *et al.*, 1998). In addition, Durica *et al.* (2002) found coexpression of the two receptors UpEcR and UpRXR in the ovary of the crab, *Uca pugilator* during ovarian development suggesting that ovary is a potential target for ecdysone action. However, it is not clear whether this receptor activity is related to Vg synthesis in the ovary.

Furthermore, Tarrant *et al.* (2011) reported on the occurrence of EcR/RXR isoforms in the American lobster *Homarus americanus* and correlated their expression pattern with the regulation of ovarian development. Subsequently, Mu *et al.* (2014) found higher level of EcR expression in the ovaries of the crab, *Portunus trituberculatus* after copulation indicating that ecdysteroids have a role in ovarian maturation. EcR isoforms have also been isolated from other crustacean species such as *Marsupenaeus japonicus* (Asazuma *et al.*, 2007), *Macrobrachium nipponense* (Shen *et al.*, 2013), and the blue crab *Callinectes sapidus* (Techa and Chung, 2013). In the mud crab, *Scylla paramamosain*, Gong *et al.* (2015) reported three isoforms of ecdysone receptors (*SpEcR*) in the ovary, suggesting a possible role in promoting ovarian development. In addition, incubation

of previtellogenic ovarian explants with 20 hydroxyecdysone induced increased expression of *SpEcR* as well as vitellogenin (SpVg). Interestingly, *in situ* hybridization studies indicated that *SpEcR* mRNA was present only in the follicular cells and not in the oocyte. In the Chinese mitten crab, *Eriocheir sinensis*, Yang *et al.* (2007) showed that follicle cells play a major role in the transport of nutrient reserves into the oocytes when ovarian maturation begins. Uptake of vitellogenin from the hemolymph by receptor-mediated endocytosis is an important process in the vitellogenesis of the mud crab *Scylla serrata* (Warrier and Subramoniam, 2002). Conceivably, ecdysteroids could mediate the uptake of vitellogenin into the oocytes in the brachyuran crabs, and other decapod crustaceans, in which edysteroid receptors have been localized in the ovary.

In the above studies, EcR activity has been demonstrated only in the ovary. However, Girish *et al.* (2015) found increased expression of RXR and EcR in the hepatopancreas of the freshwater crab *Oziothelphusa senex senex*, followed by upregulation of ecdysone-responsive gene E75 during vitellogenic stages I and II. These authors suggest that RXR, EcR and the ecdysone responsive gene E75 are involved in the regulation of synthesis of vitellogenin in hepatopancreas, whereas in ovary, they may play an important role in the uptake of Vg from the hemolymph by regulating the levels of vitellogenin receptor. Interestingly, in a primitive cladoceran *Daphnia magna*, Tokishita *et al.* (2006) reported the presence of an ecdysone-responsive element in the upstream of the Vg gene, suggesting the possible transcriptional activation of Vg gene by ecdysteroids. In addition, there is significant sequence homology between *D. magna* and insect Vgs. Thus, ecdysteroid receptor expression in the female reproductive tissues of decapod crustaceans implicates their role as hormone ligands in the stimulation of vitellogenesis. Furthermore, the discovery of ecdysone-responsive elements in the primitive crustacean species underscores the convergence in the evolution of endocrine mechanisms controlling reproduction in insects and crustaceans.

Role of Vertebrate-type Sex Steroids

In vertebrates, sex steroid hormones such as progesterone, estradiol and testosterone are

synthesized in the gonads, playing several physiological roles in growth, development and reproduction (Wierman, 2007). In oviparous vertebrates like fishes, it has been clearly established that Vg gene expression is regulated by estradiol-17 β via estrogen receptor (Pakdel *et al.*, 1991). Recent studies have uncovered the occurrence of vertebrate-type sex steroids in a variety of invertebrate forms (Lafont and Mathieu, 2007). But in most cases, evidence of their endogenous origin and the involvement in endocrine regulations has not been demonstrated, as in vertebrates.

Crustaceans are of particular interest in the steroid hormonal control of reproduction in as much as the egg maturation under controlled condition assumed greater importance in the aquaculture of commercially important shrimp species all over the world (see Subramoniam, 2016 for a discussion). As yet, the reported occurrence and endogenous synthesis of several steroidal compounds in the reproductive tissues of decapod crustaceans posit the possible existence of a steroid-based endocrine system in Crustacea (Subramoniam, 2000).

Employing gas chromatography/mass spectrometry (GC/MS) with selected ion monitoring (SIM), Fairs *et al.* (1989) confirmed the presence of 17 β -estradiol in the eggs and hemolymph of the lobster *Nephrops norvegicus*. Similarly, in the ovary of *Penaeus monodon*, and the crab *Scylla serrata*, both conjugated and nonconjugated steroidal compounds have been found to accumulate during the progression of vitellogenesis (Fairs *et al.*, 1990; Warrier *et al.*, 2001). Steroidogenesis of these hormones in the ovary and hepatopancreas was subsequently demonstrated in the crab *Carcinus maenas* and the fresh water prawn *Macrobrachium rosenbergii* (Hazel, 1986; Ghosh and Ray, 1993).

Fluctuations in the levels of sex steroids in the hemolymph, correlated to ovarian activity in lobsters suggest that estradiol may have a role in vitellogenesis in crustaceans (Couch *et al.*, 1987). A marked association between vitellogenesis and estradiol levels has also been indicated in the anostracan *Artemia* (Van Beek and De Loof, 1988). Subsequently, titer changes in the steroid hormones have been reported in several crustacean species (Table 1). In the penaeid shrimp, *P. monodon*, 17 β -estradiol and progesterone levels in the ovary, hepatopancreas, and hemolymph

fluctuated closely with those of vitellogenin during ovarian cycle (Quinitio *et al.*, 1991). Eyestalk ablation resulted in the increase in the levels of estradiol and progesterone in the hemolymph, ovary and hepatopancreas of this shrimp (Merlin *et al.*, 2016). Similar fluctuations of these hormones in the ovary, hepatopancreas and the hemolymph during vitellogenesis have been observed in many other decapods such as crabs (Warrier *et al.*, 2001), shrimps (Yano, 2000) and crayfish (Coccia *et al.*, 2010). In the crab *Scylla serrata*, the progesterone level is always higher than that of estrogen, especially in the late vitellogenic stage. In vertebrates, progesterone regulates both sexual differentiation and sexual reproduction, besides being a precursor for other steroid hormones including estradiol, which induces vitellogenesis in fish and amphibians (Nagahama, 1983). In crustaceans, progesterone is suggested to control vitellogenesis and oocyte maturation. Interestingly, in *M. rosenbergii*, hormonal fluctuations were found only during the reproductive molt cycle, whereas in the non-reproductive molt cycle, characterized by undeveloped ovary, the levels were either low or undetectable (Gunamalai *et al.*, 2006). These studies further indicate a role for vertebrate steroids in the control of vitellogenesis in Crustacea.

Experimental evidence for the influence of vertebrate-type sex steroids was provided in several shrimp species in which injection of estrogen and progesterone resulted in the increased release of vitellogenin into the haemolymph (Yano, 1985, 1987). *In vitro* culture of previtellogenic ovary of *M. japonicus* with 17β -estradiol also elevated vitellogenin synthesis (Yano and Hoshino, 2006). Furthermore, Coccia *et al.* (2010) noticed pronounced vitellogenin (Vg) mRNA synthesis in the early vitellogenic ovary of the crayfish *Cherax albidus* injected with 17β -estradiol and progesterone. In a more recent study on the penaeid shrimp, *P. monodon*, Merlin *et al.* (2015) reported that both estrogen and progesterone injection stimulated Vg mRNA expression in the ovary and hepatopancreas during previtellogenic stage. In the freshwater crab, *Oziothelphusa senex senex*, administration of 17β -estradiol and progesterone increased ovarian index, oocyte diameter and ovarian vitellin levels (Swetha *et al.*, 2016). Taken together, these results suggest that the vertebrate-type sex steroids are not only synthesized endogenously in crustacean reproductive tissues, but also modulate

vitellogenin synthesis in ovary and hepatopancreas.

In oviparous vertebrates, hormonal regulation of vitellogenin gene expression is achieved by the binding of estrogen to its nuclear receptor, which binds to estrogen responsive elements in the promoter region of the Vg gene (Tata and Smith, 1979). In the frog, *Xenopus*, vitellogenin gene expression is controlled by panoply of hormones, including estrogen, progesterone, thyroid hormone and prolactin, with thyroid hormone potentiating estrogen activation of vitellogenin gene in the hepatocytes (Rabelo and Tata, 1993). In Crustacea, several recent studies have indicated the expression of receptors of estrogen and progesterone both in the ovary and hepatopancreas (see Table 1). For example, in *Penaeus monodon*, Merlin *et al.* (2015) found a simultaneous expression of the receptors of estrogen and progesterone in the ovary, adducing further evidence that these hormones mediate Vg gene expression in penaeid shrimp. Interestingly, the ovarian response to these hormones is more pronounced in the eyestalk ablated females than the unablated adults. The inference is that the suppressive effects of VIH on Vg synthetic system in decapod crustaceans is more overpowering than the stimulatory effects of vertebrate steroids.

Vertebrate sex steroids could also exert their influencing effect on vitellogenesis via other hormone signaling pathways such as ecdysteroids. In the freshwater field crab, *O. senex senex*, administration of progesterone and 17β -estradiol significantly increased the mRNA levels of ecdysteroid receptor and retinoid receptor in the hepatopancreas and ovary. This in turn resulted in increased synthesis of Vg mRNA in ovary and hepatopancreas (Swetha *et al.*, 2016). At the present, it is not clear whether ecdysteroids and vertebrate steroids have a combined effect on the stimulation of vitellogenin synthesis in crustaceans. But the pattern of receptor expression of these steroid hormones suggests a possibility.

In vertebrates, the synthesis of the ovarian steroid hormones is stimulated by the gonadotropic hormones such as follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Weghofer *et al.*, 2007). The existence of GnRH-like and gonadotropin (GTH)-like hormones has been demonstrated in the brain and thoracic ganglia of several crustaceans (Saetan *et al.*, 2013; Tinikul *et al.*, 2011; Huang *et al.*, 2008; Ye

Table 1: Vertebrate steroids in crustacean having a role in ovarian maturation

Steroids	Species	Techniques to study the hormonal effect	Organs taken for analysis	Criteria used in functional implication	References
Estradiol (E2) and progesterone (PG)	<i>Penaeus monodon</i>	Enzyme Linked Immunosorbent Assay (ELISA) Histology & Immunohistochemistry	Hemolymph, ovary and hepatopancreas Ovary	Relatively higher level of E2 and PG found in hepatopancreas at all ovarian stages Estrogen receptor and progesterone receptor protein signals found in pre-vitellogenic oocytes of immature ovary	Merlin <i>et al.</i> , 2016
17 β -Estradiol(E2)& Progesterone(PG)	<i>Oziothelphusa senex senex</i>	Histological studies ELISA RT-PCR In-silico analysis	Previtellogenic ovary Previtellogenic ovary Hepatopancreas, Previtellogenic ovary Previtellogenic ovary	Ovarian index and oocyte diameter increased after injection Ovarian vitellin level increased after injection 1. Vg mRNA level in the hepatopancreas found increased after injection.2. mRNA level of ecdystone receptor(EcR) and retinoid receptor increased in hepatopancreas and ovary after injection Interaction between E2, PG and ecdysteroid receptor suggests the mediation of EcR in inducing vitellogenesis after injection	Swetha <i>et al.</i> , 2016
17 α -hydroxy progesterone (17 α -OHP) and 17 α -hydroxy pregnenolone (17 α -OHPL)	<i>Scylla olivacea</i>	Histological studies	Immature crab in intermolt stage	Crab injected with 17 α -OHPL showed highest Gonadosomatic index(GSI) and large oocytes compared with 17 α -OHP	Muhd-Farouk <i>et al.</i> , 2016
17 β -Estradiol(E2)	<i>Penaeus monodon</i>	Histological analysis	Immature(white) immature (Yellow) Vitellogenic ovary	E2 exposure in culture facilitates oocyte enlargement	Merlin <i>et al.</i> , 2015
17 α -hydroxy progesterone (17 α -OHP)			Immature(white), Vitellogenic ovary	17 α -OHP in culture facilitates oocyte enlargement	
		RT-PCR	Immature (Yellow) Vitellogenic ovary	E2 in culture stimulates Vitellogenin(Vg) mRNA synthesis 17 α -OHP in culture stimulates Vg mRNA synthesis	
		Gonadosomatic index (GSI)	Immature ovary	E2 and 17 α -OHP treatment along with eyestalk ablation resulted in higher GSI	
		RT-PCR	Immature ovary	Vg mRNA transcript level found higher for the animal which injected with both 17 α -OHP and eyestalk ablation	
		Western blotting	Immature(white), immature (Yellow), Vitellogenic ovary	Estrogen receptor (ER) and progesterone receptor(PR) expression in the ovary	
17 α -Hydroxy-Progesterone	<i>Neohelice granulata</i>	Gonadosomatic index	Post-reproductive period ovary	Gonadosomatic index increased significantly in females fed with pelleted feed	Medesani <i>et al.</i> , 2015
		ELISA	Post-reproductive period ovary	Vitellogenic proteins (Vg) content in the ovary found increased after feeding with pelleted feed	

Table 1: Contd....

Steroids	Species	Techniques to study the hormonal effect	Organs taken for analysis	Criteria used in functional implication	References
		Protein synthesis assay	Post-reproductive period ovary	Ovarian protein level in culture found increased	
		Vitellogenic protein concentration assay	Post-reproductive period ovary	Vitellogenic proteins (Vg) content in the cultured ovary increased	
17 β -oestradiol (E2) and 17 α -hydroxy progesterone (PG)	<i>Astacustepto-dactylus</i>	Radioimmunoassay (RIA)	Hemolymph, ovary and hepatopancreas	E2 level increased in the haemolymph, ovary and decreased in the hepatopancreas during vitellogenesis, PG level enhanced in hemolymph and ovary during maturation stage	Malati <i>et al.</i> , 2013
17 β -Estradiol(E2) Progesterone(PG)	<i>Cherax albidus</i>	Histological analysis	Hepatopancreas	Hepatopancreas morphology changed during early and late vitellogenic females after injection. Increase in the size of the hepatopancreatic cells mainly due to the presence of large lipid vacuoles	Coccia <i>et al.</i> , 2010
		Immunohistochemistry	Hepatopancreas	In Early vitellogenic females, Vtg immunoreactivity found in the vacuoles of some epithelial cells after injection	
		RT-PCR	Hepatopancreas	E2 found to be more effective than progesterone on Vtg mRNA synthesis in the hepatopancreas after injection	
		Western blotting	Hemolymph	PG was more effective than E2 and E2 plus PG in increasing the vitellogenin concentration in the hemolymph of Early vitellogenic and Full vitellogenic females under injection	
Progesterone (PG)	<i>Scylla paramamosain</i>	ELISA	Hemolymph, ovary and hepatopancreas	Peak levels of PG detected during previtellogenic stage in hemolymph, ovary, and hepatopancreas. PG level decreased significantly in vitellogenic stage I. During vitellogenic stage II, progesterone levels rose again in the hemolymph and ovary, but continued to decrease in the hepatopancreas	Ye <i>et al.</i> , 2010
		Western blotting	Ovary	Progesterone receptor identified in vitellogenic ovary I & II	
		Immunohistochemistry	Ovary	PR was detected mainly in the follicle cells during vitellogenic stage I and in the nuclei of oocytes in vitellogenic stage II	
17 β -estradiol E2), testosterone (T) and 17 α -hydroxy progesterone (17-OHP; conjugated and unconjugated)	<i>Macrobrachium rosenbergii</i>	Solid-phase radioimmunoassay (RIA)	Hemolymph, ovary and hepatopancreas	High levels of unconjugated 17-OHP, relatively constant concentrations of unconjugated T, and null concentration of unconjugated E2 were found in the hemolymph, throughout the five stages of ovary	Martins <i>et al.</i> , 2007

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Steroids	Species	Techniques to study the hormonal effect	Organs taken for analysis	Criteria used in functional implication	References
17 β -Estradiol (E2)	<i>Marsupenaeus japonicus</i>	Histological studies	Previtellogenic (immature)	E2 in culture induces the appearance of primary vitellogenic oocytes in the immature ovary	Yano and Hoshino, 2006
		Rocket immunoelectrophoresis	Previtellogenic (immature) ovary	Induction of Vg synthesis and secretion in the immature ovary under cultured condition	
Estradiol-17 β (E2) and Progesterone(PG)	<i>Emerita asiatica</i> & <i>Macrobrachium rosenbergii</i>	Radioimmunoassay (RIA)	Hemolymph	E2 and PG level found high in crabs with mature ovaries	Gunamalai <i>et al.</i> , 2006
			Ovary, Hepatopancreas, Hemolymph	During the reproductive molt cycle of <i>Macrobrachium rosenbergii</i> , the level of E2 and PG in all tissues peaked during intermolt, but declined drastically at premolt and postmolt stages. The level of E2 and PG in hemolymph was not detectable in any molt stage during the non-reproductive molt with the ovary containing undeveloped oocytes	
Progesterone and Estradiol receptors (PR and ER)	<i>Austropotamobius pallipes</i>	Immunohistochemistry and western blotting	Ovary, hepatopancreas	ER found in the hepatopancreas and PR found in both the hepatopancreas and ovary after injection	Paolucci <i>et al.</i> , 2002
17 β -Estradiol(E2) Progesterone(PG)	<i>Scylla serrata</i>	Radioimmunoassay (RIA)	Ovary, hepatopancreas and hemolymph	E2 is high in hepatopancreas and PG is maximal in ovary during vitellogenic stage I	Warrier <i>et al.</i> (2001)
17 β -Estradiol(E2)	<i>Macrobrachium rosenbergii</i>	Enzyme assay	Ovary, hepatopancreas	The activity of 17 β -hydroxysteroid dehydrogenase, a key enzyme in steroid metabolism, increased after injection	Ghosh and Ray, 1993
		Radioimmunoassay	Hemolymph, ovary	E2 level in the maturing prawn increased after injection	
Progesterone-and Estradiol-17 β -like substances	<i>Pandalus kessleri</i>	Histological studies Radioimmunoassay (RIA)	Ovary and hepatopancreas	GSI, HSI significantly increased when ovaries were composed of yolky oocytes and decreased sharply after spawning	Quinitio <i>et al.</i> , 1991
			Hemolymph	Progesterone level found increased at the onset of vitellogenesis and decreased during vitellogenesis. Estradiol concentrations rose during the peak of vitellogenesis	
Estradiol 17 β (E2) and Progesterone(PG)	<i>Homarus americanus</i>	Radioimmunoassay	Mandibular organ, green gland, hepatopancreas, ovary and serum	E2 and PG found undetectable in all tissues of animals possessing immature ovaries, except in the mandibular organ. PG concentration was almost identical in mandibular organs of all animals with developing ovaries	Couch <i>et al.</i> , 1987
17 β -Hydroxy- Progesterone	<i>Penaeus Japonicus</i>	Rocket immunoelectrophoresis	Early vitellogenic ovary	Stimulation of vitellogenin synthesis and release into the hemolymph after injection	Yano, 1987

et al., 2011). Interestingly enough, bursicon, an invertebrate heterodimeric glycoprotein, which is structurally similar to vertebrate gonadotropins, is also present in crustaceans (Chung *et al.*, 2012). Bursicon, synthesized mainly in thoracic ganglia, plays a major role in cuticle tanning during molt cycle of crustaceans (Webster *et al.*, 2013). Recently, Sathapondecha *et al.* (2015) reported that the treatment of primary ovarian cells with the heterodimeric bursicon, r bursa $\alpha\beta$, elevated Vg mRNA expression two-fold in *P. monodon*, compared to the control. Although the exact mechanism of bursicon action on vitellogenin synthesis has not been determined, it could possibly stimulate steroidogenesis, in much the same way the gonadotropins stimulate gonadal activity in vertebrates (Erickson and Hsueh, 1978).

In vertebrates, the pituitary-derived gonadotropins, FSH and LH regulate the expression and activity of key steroidogenic enzymes which stimulate gonadal development and maturation. In teleosts, FSH regulates initial vitellogenesis in ovaries where estrogen, produced in the ovarian follicles by aromatization of androgen (by aromatase, *cyp19*), stimulates liver to produce vitellogenin which is sequestered by the oocytes (Nagahama, 1994). The sex steroids exert positive or negative feedback on the brain and pituitary depending on the maturational stage, modulating the expression of reproduction related neuropeptides and neurotransmitters (Zohar *et al.*, 2010). Among invertebrates, aromatase activity has been demonstrated only in mollusk by the detection of cytochrome P450 aromatase in the freshwater snail, *Biomphalaria glabrata* (Lockyer, *et al.*, 2005). In the scleractinian coral *Euphyllia auccora*, Twan *et al.* (2006) demonstrated aromatase hormone expression in the polyp tissue and implicated its activity in the conversion of testosterone into estrogen. Among crustaceans, aromatase activity has been shown in the hepatopancreas of the penaeid shrimp *Marsupenaeus japonicus* (Summavielle *et al.*, 2003). During vitellogenesis, testosterone, produced in the ovary, is transported to hepatopancreas and metabolized into 17 β -estradiol by aromatase enzyme. Evidently, the endogenously produced sex steroids could induce vitellogenesis in this shrimp.

The presence of different vertebrate gonadotropin-like glycopeptides in crustaceans, together with the steroidogenic ability of the ovary

and hepatopancreas, lends support to the contention that a steroidal control of vitellogenesis would be operative in the crustaceans too. Pooling information on multifarious molecules purported to be involved in the control of oocyte maturation in crustaceans, a possible networking of interactive hormonal effectors to control the brain-ovary axis is proposed (Fig. 1).

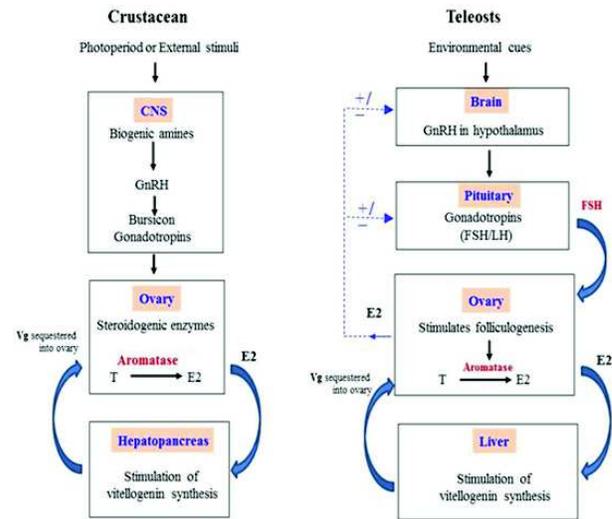


Fig. 1. Schematic graphical diagram of the pathway regulating vitellogenesis in crustaceans and teleosts. CNS- central nervous system; GnRH-Gonadotropin-releasing hormone; T-Testosterone; E2- 17 β Estradiol; Vg-vitellogenin; \pm -feedback; FSH-follicle stimulating hormone; LH-luteinizing hormone (Modified from Subramoniam, 2016)

Working of a linear hormone signaling cascade, starting with the perception of environmental cues by the biogenic amines, ending with the ovary to stimulate steroidogenesis of estrogen and progesterone is possible in Crustacea. These steroids then could control vitellogenin synthesis and associated lipogenesis in the hepatopancreas and ovary of decapod crustaceans. Incidentally, VIH does not seem to interlude in this hormonal signaling pathway, with its direct inhibitory action through second messenger system on the transcriptional activation of vitellogenin synthesis in the ovary or hepatopancreas (Subramoniam, 2011). Clearly, more work is needed to fill the gap in the brain-gonad axis of crustaceans to gain a better understanding on steroid hormone signaling pathway to control vitellogenesis as well as meiotic maturation and possibly spawning. Nevertheless, our current understanding on the

hormonal control of vitellogenesis by eyestalk hormones and the vertebrate sex steroids is adequate enough to develop protocol for the control of female reproduction in the commercially significant shrimp species.

Discussion and Conclusion

It is now well known that steroid molecules exist in almost all invertebrate phyla. Two major kinds of steroids with hormonal function are known among the invertebrates: the 'native' arthropod steroid hormone, ecdysteroids, and the 'naturalized' vertebrate-type sex steroids. While ecdysteroids have the role established in the control of molting, vertebrate sex steroids are involved in the control of gametogenesis. By far, the role in the control of ovarian maturation has been demonstrated for estrogen and progesterone mainly in mollusks. However, in mollusks, more attention has been directed at the process of sex reversal and its steroidal endocrine control (Lafont and Mathieu, 2007). Nevertheless, evidence for functional significance of the sex steroids in the control of vitellogenesis in crustaceans is steadily mounting. In crustaceans, yolk precursor protein synthesis is hormonally regulated. In penaeid shrimps, both ovary and hepatopancreas equally contribute to the synthesis of yolk. However, in other decapods, hepatopancreas is the sole site of vitellogenin synthesis (Subramoniam, 2011). As discussed above, estrogen and progesterone show correlative fluctuations in the hemolymph, ovary and hepatopancreas with different phases of vitellogenesis. *In vivo* injection of these hormones also stimulated vitellogenin synthesis in ovary and hepatopancreas. In the penaeid shrimps, expression of receptors for these hormones coincides with increased Vg mRNA expression, suggesting further evidence that vertebrate steroids initiate vitellogenin gene expression in the ovary (Merlin *et al.*, 2015). That the hepatopancreas also responds to sex steroids has been revealed by the expression of their respective receptors during vitellogenesis in the fresh water crayfish *Austropotamobius pallipes* (Paolucci *et al.*, 2002). Thus, evidence is in favor of the contention that estradiol and progesterone control the transcriptional activation of vitellogenin gene in Crustacea. In addition, sex steroids, especially progesterone may have a role in post vitellogenic processes such as meiotic maturation and spawning,

suggesting that steroids may act on multiple steps of female reproduction in crustaceans.

The fact that ecdysteroids are in circulation during the vitellogenic cycle in shrimps and prawns indicates a role in the control of yolk formation for the molting hormone in crustaceans too. Ecdysteroid receptor, along with its dimerizing partner, RXR, is also shown to be expressed in the ovary (especially, follicle cells) during vitellogenesis in crabs, shrimps and prawns, suggesting that ecdysone has a combined role with sex steroids in the control of vitellogenic activities. The reported occurrence of an estrogen responsive element in the upstream of Vg gene in the primitive cladoceran, *Daphnia* lends further support to this possibility. Understandably, steroidal control of vitellogenesis in the crustaceans show similarity with that in oviparous vertebrates in as much as transcriptional activation of vitellogenin gene is accomplished by sex steroids. Conversely, the role of ecdysteroids in the control of vitellogenesis in crustaceans points to the phylogenetic relationship with the insects.

Interestingly, in insects, the sex steroids do not have any role in the control of vitellogenesis, although their occurrence has been reported in some insects. The hormonal control of vitellogenesis in insects has been taken over by two native arthropod hormones, viz., Juvenile Hormone III and 20-hydroxyecdysone. It is only in Crustacea that steroidal control of vitellogenesis and egg maturation has assumed greater importance, although crustaceans have shared Juvenile hormone-like (methyl farnesoate) and ecdysteroid control of egg maturation with insects.

At present, eyestalk ablation is the only hormonal manipulation technique employed in induced maturation in the shrimp hatcheries. Despite its success in the seed production, the quality of the seed produced is always poor. One way to improve the quality of the seeds is by developing a protocol in which eye stalk ablation technique could be combined with sex steroid hormone treatment for induced maturation and spawning in the shrimps. By this method, greater success could be achieved in the production of healthy, quality shrimp seeds under hatchery conditions. Crustacean lipovitellin has high lipid content (up to 30%) and hence the lipid content of the egg could determine the quality of the seed produced. In this context, estrogen has been shown

to have stimulatory effect on the synthesis of two lipogenic enzymes, viz., malate dehydrogenase and glucose-6-phosphate dehydrogenase in the hepatopancreas of the fresh water prawn, *Macrobrachium rosenbergii* during vitellogenesis (Ghosh and Ray, 1994). Thus, vertebrate sex steroids would have an important role in bolstering the healthiness of the seeds produced by hormonal manipulation techniques. Yet again, the purported role of progesterone in inducing meiotic maturation and spawning would add to the overall suitability of vertebrate sex steroids in the protocol development for induced maturation of commercially important shrimps.

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