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Impact of 17α- Hydroxy-Progesterone and Eyestalk Ablation on Ovarian Maturation in Relation to Protein Changes in the Ovary and Hepatopancreas in Freshwater Crab, *Barytelphusa cunicularis*

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ABSTRACT

The present work was planned to determine the effect of 17α-hydroxy progesterone hormone and eyestalk ablation on ovarian maturation in relation to protein changes in the ovary and hepatopancreas of freshwater crab, *Barytelphusa cunicularis*. The studies showed that, administration of 17α-hydroxy progesterone hormone indicated considerable changes in protein content during ovarian maturation over base control and experimental control crabs. In the present examination eyestalk ablation showed increase in ovarian maturation over base control, experimental control and hormone treated crabs by elevating the mobilization of biochemical constituent(s) from hepatopancreas to the ovary. Moreover, eyestalk ablation method found more significant than hormone injected which may be used for enhancement of crustacean culture.

KEY WORDS: *BARYTELPHUSA CUNICULARIS*, OVARIAN MATURATION, PROTEIN, 17α-HYDROXY PROGESTERONE

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INTRODUCTION

Population explosion, pollution, climate change, unplanned management and other factors increases pressure on the agricultural productivity which reduces access to food quantity and quality. So to fulfill the need of the people, aquaculture now a day's is a good and reliable source particularly crustaceans; which is an important source of aquatic food protein, ornamental and trade which are extremely important for any developing countries. It provides both economic development and empowerment in terms of contribution to GDP, consumption, employment, catch value and exports. The crustacean sector generates high value export products which enables producers to buy lower value products in the world market which is a positive contribution to food security in both producing and exporting countries (Bondad-Reantaso et al. 2012). Enhancement of aquaculture entirely depends upon reproduction which is the most fundamental and essential high energy demanding physiological process in living organisms to continue its race in which gonad maturation plays a vital role; whereas some species require external manipulation for it. The gonadal development can be stimulated or inhibited by affecting the neurosecretory cells by many pharmacological agents and eyestalk ablation (Subramoniam 2000; Lafont and Mathieu 2007; Coccia et al. 2010; Alejandra et al. 2011,Yang Lu et al 2018,Raghavan and Ayanath 2019).

Likewise, steroid hormones are biologically active in crustaceans found in the hepatopancreas, ovary and haemolymph which control vitellogenesis (Stevenson et al. 1979; Quackenbush 2001). Vitellogenesis is an important physiological process associated with female reproduction, because the primary source for the developing crustacean embryo is yolk protein and is a pivotal stage during crustacean reproduction. It is the synthesis of yolk proteins i.e. Vitellin (Vn) and Vitellogenin (Vg) which are the two main yolk proteins, that are important nutritive sources, which are necessary for the proper maturation and development of the oocytes (Tseng et al. 2001; Zapata et al. 2003). In decapod crustaceans, hepatopancreas acts as center for storage and / or synthesis of biochemical material which is transferred to sites of gametogenesis for the purpose of growth, maintenance and reproduction (Adiyodi and Adiyodi 1970). The mobilization and accumulation of protein, lipid and glycogen reserves in several tissues have been documented in several crustacean species (Khayat et al. 1994; Harrison 1997; Tseng et al. 2001; Thomas et al. 2005). In this regard the researchers have reported the role of some vertebrate-type steroids such as β- estradiol and progesterone in ovarian maturation. Reddy et al. (2006) demonstrated that 17α- hydroxyprogesterone hormone induced ovarian growth and ovarian VTG synthesis in the fresh water crab, *Oziotelphusa senex senex*.

Muhd-Farouk et al. (2014) studied effect of vertebrate steroid hormones on the ovarian maturation stages of orange mud crab, *Scylla olivacea* and found enhancement in the ovarian maturation. Sujathamma and Dayakar (2015) observed effect of estradiol and 17α-hydroxyprogesterone on ovarian development of fresh water paddy field crab, *Oziotelphusa senex senex* and found that both hormones were influencing the gonadal growth. Kale (2017) found significant ovarian maturation by mobilization of protein from hepatopancreas to ovary under the influence of α- estradiol hormone and eyestalk ablation in fresh water crab, *B. cunicularis*. Yang Lu et al. (2018) examined the effect of exogenous estrogen on the ovarian development and gene expression in the female swimming crab.

Portunus trituberculatus and found that hormone injection probably indirectly stimulate the ovarian development and vitellogenesis by mediating the secretion of hormones and gene expression in the endocrine organs. Raghavan and Ayanath (2019) investigated the effect of ecdysteroids on oogenesis in the freshwater crab, *Travancoriana schirnerae* and found positive results. The eyestalk is also one of the critical factors know n to control crustacean reproduction by a series of inhibitory neurosecretory factors which effectively targets the ovaries and hepatopancreas (Laufer et al. 1998; Aktas et al. 2003). Hussain et al. (2014) studied the effect of unilateral eyestalk ablation in fresh water

prawn, *Macrobrachium lamarrei* and noticed to induce gonadal development. Samyappan et al. (2015) studied impact of unilateral eyestalk ablation on lipid profiles in fresh water female crab, *Oziotelphusa senex senex* which showed a marked decrease in the hepatopancreas and a significant increase in ovarian tissue indicating ovarian maturation. Sarojini et al. (2016) studied impact of unilateral eyestalk ablation on protein content in freshwater crab, *Spiralothelphusa hydrodroma* and found significant increase in ovarian maturation. Recently, Rana (2018) revealed that eyestalk ablation is the alternative technique in induced breeding for rapid ripening of ovaries to enhance meat yield in aquaculture in freshwater crab, *Barytelphusa lugubris*. Available literature states that ovarian maturation is the process in which there is intense requirement of biochemical constituents like protein, lipid and glycogen which are transported from the storage organs under controlled hormone action. Present investigation was undertaken to confirm whether 17α-hydroxy progesterone hormone enhance the ovarian maturation in relation to synthesis and mobilization of protein from hepatopancreas to ovary and the results were compared with eyestalk ablation.

MATERIALS AND METHODS

Barytelphusa cunicularis were collected from Godavari River near Newasa and were acclimatized for a week in the laboratory. Healthy well-adapted female crabs of early-reproductive phase of approximately same weight and size of intermoult stage ranging between 35-40g body weights were selected for the experiments. Water in the troughs was changed daily and crabs fed by small pieces of earthworm and bivalve on alternate days. Other parameters like temperature, pH, salinity, photoperiod etc., were maintained constant during experiment. The crabs were divided into four groups, as base control, experimental control, 17α-hydroxy progesterone injected and eyestalk ablated group, each containing 10 crabs. The final concentration of the hormone preparation was 1µl = 1µg. The hormone was injected into the abdominal musculature of arthropodial membrane through 3rd walking legs, receiving a dose of 20µl /crab with the help of hypodermic syringe having a 27-guaze needle.

Preparation of 17α- hydroxy progesterone hormone injection: 10mg of 17α-hydroxy progesterone hormone was dissolved in 1ml of 1% ethanol and resulting solution diluted to 10ml by adding glass distilled water. The final concentration of the hormone preparation was 1µl = 1µg. From this preparation hormone was injected to crab receiving a dose of 20µl hormone/crab. The used hormone was obtained from Sigma Chem. (USA). The experiment was conducted for a period of 21 days; base control crabs were sacrificed on o-day of the experiment and remaining grouped crabs were sacrificed on 21st day of the experiment. Ovaries and hepatopancreas were dissected out for estimation of protein levels by Lowry et al. (1951). Protein levels were calculated on dry weight basis and expressed as % mg.

RESULTS AND DISCUSSION

Protein levels in the ovary were found to be 28.81 %, 31.72 % and 45.12 % for base control, experimental control and 17α-hydroxy progesterone injected respectively. Eyestalk ablated crabs showed 50.38 % protein level. While protein content in hepatopancreas were noted to be 48.12 %, 47.19 % and 36.47 % for base control, experimental control and 17α-hydroxy progesterone injected crabs respectively. Eyestalk ablated crabs showed 26.98 % of protein level. Increased protein level in eyestalk ablated group was prominent when compared to protein levels obtained in hormone injected. Biochemical studies are very important from the reproductive as well as the nutritive point of view in any edible crustacean animals particularly protein content. Protein plays very important role in the growth and maintenance of the animals and it is easily digestible too. In the present study (Table 1 and Fig. 1) the protein levels in the ovary were found to be 28.81%, 31.72% and 45.12% for base control, experimental control and 17α-hydroxy progesterone injected respectively. Whereas eyestalk ablated crabs ovary showed 50.38% protein level. While protein content in hepatopancreas were noted to be 48.12%, 47.19% and 36.47% for base control, experimental control and 17α-hydroxy progesterone injected crabs respectively. Eyestalk ablated crabs showed 26.98% of protein level. The present findings

indicated that hormone injections and eyestalk ablation showed enhancement in the ovarian maturation by synthesizing or and mobilization of the protein and other biochemical constituents from hepatopancreas to ovary over the base control and experimental control which seems reasonable that decreases were a consequence of accelerated transport to maturing ovaries in which proteins and lipids are accumulated and hepatopancreas may be the source for these constituents circulated through the haemolymph (Kulkarni et al. 1979; Teshima et al. 1988; Okumura and Aida 2001). Similarly, the role of steroid hormones in controlling the process of reproduction has been studied by (Yano 1987; Quackenbush 1989a; Subramoniam 2017).

In the same ray of light Tsukimura (2001) studied the effect of 17 α-estradiol, progesterone and 17 α-hydroxyprogsterone on ovarian maturation of crustaceans and found positive correlation to protein synthesis and its uptake for the maturation in ridgeback shrimp, *Sicyonia ingentis*. Zapata et al. (2003) observed ovarian growth in the crab, *Chasmagnathus granulata* induced by 17α-hydroxyprogesterone and Juvenile hormone III. Sujathamma and Dayakar (2015) in their studies examined the effect of estradiol and 17α-hydroxyprogesterone on ovarian development of fresh water paddy field crab, *Oziotelphusa senex senex* and found that both hormones promotes the gonadal growth and reproduction in female crab. Muhd-Farouk et al. (2016) noticed that the administration of 17α-OH pregnenolone as well as 17α-OH progesterone stimulated ovarian index, oocyte diameter and ovarian maturity in *S. olivacea* Kale (2017) studied the influence of β- estradiol hormone and eyestalk ablation on protein metabolism in fresh water crab, *Barytelphusa cunicularis* and found ovarian maturation due to mobilization of protein from hepatopancreas to ovary. Recently, Raghavan and Ayanath (2019) investigated effect of ecdysteroids on oogenesis in the freshwater crab, *Travancoriana schirnerae* study indicated that 20-OH ecdysone can stimulate ovarian growth and maturation in all phases of the oogenic cycle. Present studies also reveals that decrease in protein level in hepatopancreas and increased protein level of ovary in eyestalk ablated group was prominent over hormone injected crabs.

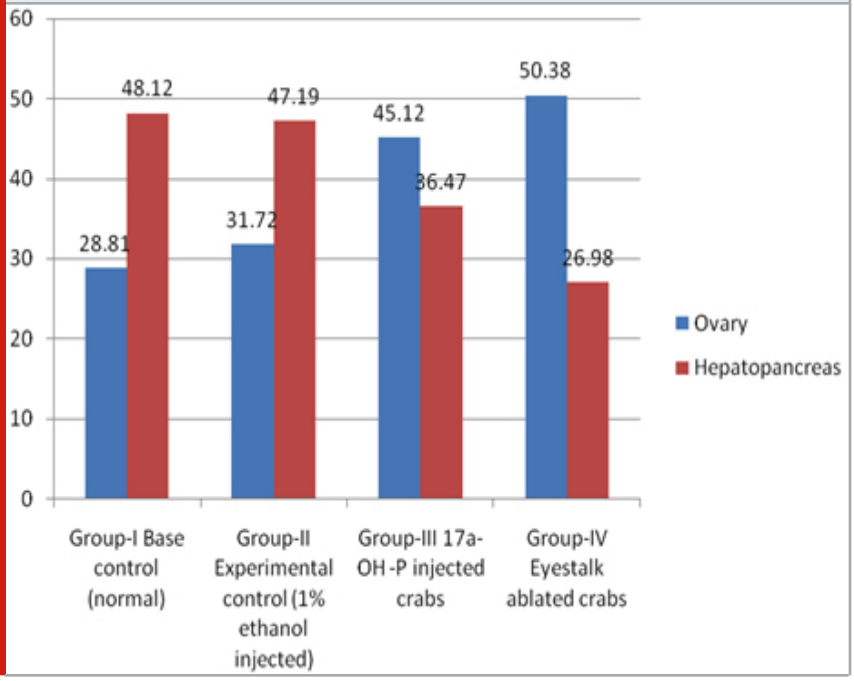
This may be due to the eyestalk ablation; because neuroendocrine system has long being known to play a significant role in gonadal maturation, and or moulting (Quackenbush 1986; Fingerman 1987). In the same way Arcos et al. (2003) reported similar results in shrimp, *Litopenaeus vannamei* suggesting decreases in total protein, cholesterol and triglycerides in haemolymph and ovarian maturation as an effect of eyestalk ablation. *Murugesan* et al. (2008) noticed eyestalk ablation influenced protein, lipid and carbohydrate content in ovarian maturation of crab, *Charybdis lucifera*.

Table 1. Shows levels of protein (% mg on dry weight basis) in ovary and hepatopancreas of different groups in freshwater crab, *B. cunicularis*.

Animal Category	No. of Crabs	Exp. Day	Tissue	Protein (%mg)
Group-I Base control (normal)	10	0-day	Ovary	28.81 ± 0.03
			Hepatopancreas	48.12 ± 0.31
Group-II Experimental control (1% ethanol injected)	10	21-day	Ovary	31.72 ± 0.34
			Hepatopancreas	47.19 ± 0.23
Group-III 17α-OH -P injected crabs	10	21-day	Ovary	45.12 ± 1.07
			Hepatopancreas	36.47 ± 0.82
Group-IV Eyestalk ablated crabs	10	21-day	Ovary	50.38 ± 0.28
			Hepatopancreas	26.98 ± 0.89

± S. D.: Mean Standard Deviation
17α-OH –P: 17 α- hydroxy progesterone hormone

Figure 1. Comparative levels of protein (% mg on dry weight basis) in ovary and hepatopancreas of different groups in freshwater crab, *B. cunicularis*



Varalakshmi and Reddy (2010) found significant changes in carbohydrate, protein and energy leading to ovarian maturation in *Macrobrachium lanchesteri* due to eyestalk ablation. Unilateral eyestalk ablation in freshwater female crabs, *Oziotelphusa senex senex* showed marked decrease in lipid classes in the hepatopancreas but significant increase in ovarian tissue which suggests ovarian maturation (Samyappan et al. 2015). Lee et al. (2017) and Guan et al.(2017) have also mentioned that eyestalk ablation is considered as the most effective method to facilitate molting and quick ovarian maturation in captive economic crustacean culture. Rana (2018) studied the effect of eyestalk ablation in freshwater crab, *Barytelphusa lugubris* and found that eyestalk ablation not only promoted the process of moulting but it also amplified the process of ovarian maturation. Similarly, Shabnam Banoo et al. (2018) also noticed increased gonadosomatic indices and gonad maturation of freshwater crab, *Paratelphusa hydrodromous* due to unilateral eyestalk ablation. Mhd Ikhwanuddin et al. (2019) found increase in gonadosomatic index and mean oocyte diameter which indicated ovarian maturation due to eyestalk ablation of blue swimming crab, *Portunus pelagicus*. But, Nagathinkal et al. (2017) studied molt and reproduction enhancement together with hemolymph ecdysteroid elevation under eyestalk ablation in the female fiddler crab, *Uca triangularis*. Their results indicate that throughout the annual cycle, both the somatic and the reproductive growth of *U . triangularis* are under the influence of inhibitory principles from the eyestalks. It is also revealing that mere deprival of the inhibitory principles does not culminate into successful vitellogenesis. Arguably, the inhibitory influence from the eyestalks could be a prerequisite for normal healthy maturation of the oocytes and spawning. In conclusion, from the available literature and results it seems that transfer of protein from hepatopancreas to ovary for its maturation was due to eyestalk ablation. It showed significant progress over experimental and hormone injected crabs which suggests that hormonal manipulation of crustacean reproduction is limited to eyestalk ablation for the induction of ovarian maturation till date and a new technological advances in such type

of hormonal manipulation and eyestalk ablation for understanding of crustacean endocrinology is essential for application of management programs for commercially important species in aquaculture.

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Conflict of Interest: There is no conflict of interest towards this manuscript.

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