

**INFLUENCE OF  $\beta$ -ESTRADIOL HORMONE AND EYESTALK ABLATION ON LIPID METABOLISM IN OVARY AND HEPATOPANCREAS OF FRESH WATER CRAB, *Barytelphusa cunicularis***

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**AUTHOR'S CONTRIBUTION**

The sole author designed, analysed, interpreted and prepared the manuscript.

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**ABSTRACT**

The present work was carried out to determine the effect of  $\beta$ - estradiol hormone and eyestalk ablation on ovarian maturation in relation to lipid changes in the ovary and hepatopancreas of fresh water edible female crab, *Barytelphusa cunicularis*. The experiment was conducted for a period of 21 days. Initially, the base control crabs were dissected on 0-day of the experiment and remaining grouped crabs were dissected on 21<sup>st</sup> day of the experiment. The studies showed influence of  $\beta$ - estradiol hormone in lipid content (30.21 %) in ovary and (25.18 %) in hepatopancreas. However, eyestalk ablated crabs ovary showed accumulated lipid levels (33.12 %) while hepatopancreas showed (24.22 %) lipid levels. Comparatively, the effect of  $\beta$ - estradiol hormone was significant over base control and experimental control crabs. But, eyestalk ablation showed increased ovarian maturation over base control, experimental control and hormone treated crabs by elevating the mobilization of lipid from hepatopancreas to the ovary for the purpose of ovarian maturation.

**Keywords:** *Barytelphusa cunicularis*;  $\beta$ - estradiol; eyestalk ablation; lipid; ovarian maturation.

**1. INTRODUCTION**

Crustaceans now a day become a good alternative source for human consumption to meet the need of food for ever increasing population. Edible crustaceans are a rich source of protein. This even contributes to help sustain the culture and trading. Moreover, this also ensures food security and in a way contributes to economic growth of a nation. Besides, obvious reasons restrain development of crustacean aquaculture the way it should have been. Such development is often crucially associated with the natural process of reproduction. The process calls for a set physiological process in any living organism.

This is crucial to help the race sustain and save itself from extinction. However, some species require external manipulation for the development of gonadal maturation. The gonadal development can be stimulated or inhibited by affecting the neurosecretory cells by many pharmacological agents and eyestalk ablation [1,2,3,4]. Steroid hormones is the important factor known to be biologically active in crustaceans found in the hepatopancreas, ovary and haemolymph which control vitellogenesis [5,6]. It has also been noticed that Vitellogenesis is an important physiological process associated with female reproduction, because the primary source for the developing crustacean embryo is yolk protein which

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is a pivotal stage during crustacean reproduction. The synthesis of two main yolk proteins i.e. Vitellin (Vn) and Vitellogenin (Vg) are important nutritive sources, which are necessary for the proper maturation and development of the oocytes [7,8]. 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 [9]. The mobilization and accumulation of protein, lipid and glycogen reserves in several tissues have been reported in number of crustacean species [10,11,7,12]. In this context, Kale et al. [13] found significant ovarian maturation by mobilization of lipid from hepatopancreas to ovary under the influence of  $17\alpha$ -hydroxyprogesterone hormone and eyestalk ablation in fresh water crab, *B. cunicularis*. Sujathamma and Dayakar [14] observed effect of estradiol and  $17\alpha$ -hydroxyprogesterone on ovarian development of fresh water paddy field crab, *Oziotelphusa senex senex* and found that both hormones were influencing the gonadal growth. Raghavan and Ayanath [15] 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 known to control crustacean reproduction by a series of inhibitory neurosecretory factors which effectively target the ovaries and hepatopancreas [16,17]. Hussain et al. [18] studied the effect of unilateral eyestalk ablation in fresh water prawn, *Macrobrachium lamarrei* and noticed induced gonadal development. Samyappan et al. [19] 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. [20] studied effect of unilateral eyestalk ablation on protein content in freshwater crab, *Spiralothelphusa hydrodroma* and found significant increase in ovarian maturation. Rana [21] 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*. The literature available suggests that ovarian maturation is the process in which there is an intense requirement of biochemical constituents like protein, lipid and glycogen which are transported from the storage organs under controlled hormone action. So, the present investigation was undertaken to test whether  $\beta$ - estradiol hormone enhance the ovarian maturation in relation to synthesis and mobilization of lipid from hepatopancreas to ovary and the results were compared with eyestalk ablation.

## 2. 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 35-40 g 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 constantly maintained during the experiment. The crabs were divided into four groups, as base control, experimental control,  $\beta$ - estradiol injected and eyestalk ablated group, each containing 10 crabs. The final concentration of the hormone preparation was  $1 \mu\text{l} = 1 \mu\text{g}$ . The hormone was injected into the abdominal musculature of arthropodial membrane through 3<sup>rd</sup> walking legs, receiving a dose of  $20 \mu\text{l}$  /crab with the help of hypodermic syringe having a 27-gauge needle.

### 2.1 Preparation of $\beta$ - estradiol Hormone Injection

10mg of  $\beta$ - estradiol 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  $1 \mu\text{l} = 1 \mu\text{g}$ . The preparation was used to inject the hormone into the crab receiving a dose of  $20 \mu\text{l}$  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 group's crabs were sacrificed on 21<sup>st</sup> day of the experiment. Ovaries and hepatopancreas were dissected out for estimation of lipid level in them. Barnes and Blackstock [22]; Sulphovanilline reagent method was used and calculations made on dry weight basis and expressed as % mg.

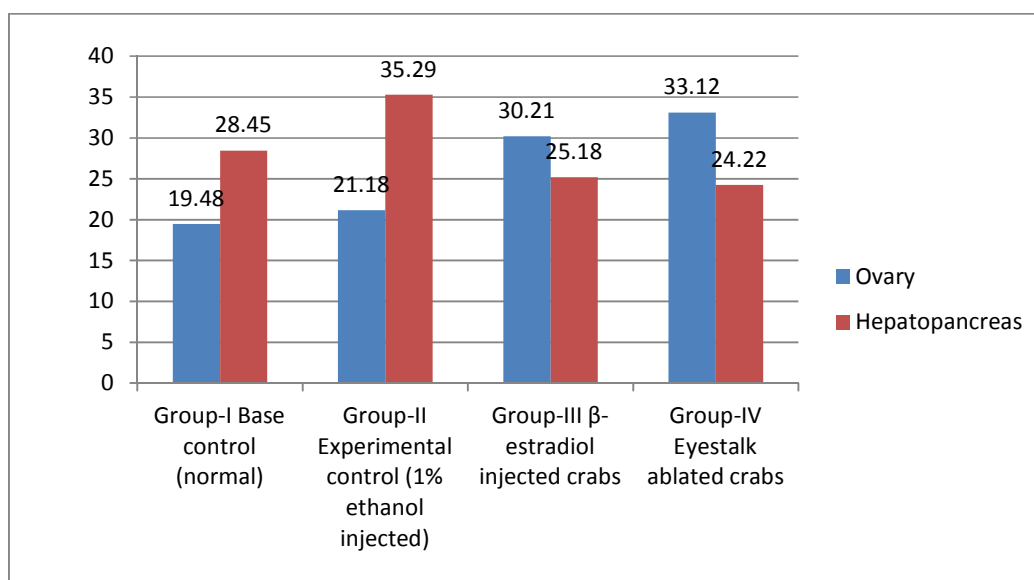
## 3. RESULTS AND DISCUSSION

Reproductive physiology in crustacean is highly controlled and governed by the nervous and endocrine systems. Specifically, oocyte maturation is a time of intense protein and lipid synthesis. Lipids serve as the major energy reserves in crustaceans and its mobilization and accumulation in different tissues also have been documented. So, biochemical studies become very important from the reproductive as well as the nutritive point of view in any edible crustacean animals chiefly the lipid content [23,24,25].

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

Animal Category	No. of crabs	Exp. day	Tissue	Lipid (%mg)
Group-I Base control (normal)	10	0-day	Ovary	19.48 ± 0.12
			Hepatopancreas	28.45 ± 0.24
Group-II Experimental control (1% ethanol injected)	10	21-day	Ovary	21.18 ± 0.10
			Hepatopancreas	35.29 ± 0.21
Group-III $\beta$ - estradiol hormone injected crabs	10	21-day	Ovary	30.21 ± 0.14
			Hepatopancreas	25.18 ± 0.19
Group-IV Eyestalk ablated crabs	10	21-day	Ovary	33.12 ± 0.15
			Hepatopancreas	24.22 ± 0.29

± S. D.: Mean Standard Deviation

**Fig. 1. Comparative levels of lipid (% mg on dry weight basis) in ovary and hepatopancreas of different groups in fresh water crab, *B. cunicularis***

In the present investigation the lipid levels in the ovary were found to be 19.48%, 21.18% and 30.21% for base control, experimental control and  $\beta$ - estradiol hormone injected respectively. Whereas eyestalk ablated crabs ovary showed 33.12% lipid level. While lipid content in hepatopancreas was noted to be 28.45%, 35.29% and 25.18% in base control, experimental control and  $\beta$ - estradiol hormone injected crabs respectively. Eyestalk ablated crabs showed 24.22% of lipid level (Table 1 and Fig. 1). Here, hormone injections and eyestalk ablation showed enhancement in the ovarian maturation by synthesizing or and mobilization of the lipid and other biochemical constituents from hepatopancreas to ovary over the base control and experimental control which seems reasonable. It's been noticed that decrease was a consequence of accelerated transport to maturing ovaries. The biochemical constituents like proteins and lipids are accumulated in the ovaries may

be circulated through the haemolymph [26,27,28]. The role of steroid hormones in controlling the process of reproduction has been reported by several authors. Tsukimura [29] studied the effect of 17  $\beta$ -estradiol, progesterone and 17  $\alpha$ -hydroxyprogesterone 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. [8] observed ovarian growth in the crab, *Chasmagnathus granulata* induced by 17 $\alpha$ -hydroxyprogesterone and Juvenile hormone III. Kale et al. [13] found accumulation of lipid in the ovaries leading to ovarian maturation under the influence of 17 $\alpha$ -OH progesterone hormone and eyestalk ablation in fresh water crab, *B. cunicularis*. Sujathamma and Dayakar [14] in their studies examined the effect of estradiol and 17 $\alpha$ -hydroxyprogesterone on ovarian development of fresh water paddy field crab, *Oziotelphusa senex senex* and found that both

hormones promote the gonadal growth and reproduction in female crab. Muhd-Farouk et al. [30] noticed that the administration of  $17\alpha$ -OH pregnenolone as well as  $17\alpha$ -OH progesterone stimulated ovarian index, oocyte diameter and ovarian maturity in *S. olivacea*. Kale [31] studied the influence of  $\beta$ - 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 [15] 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.

The present study also reveals that decrease in lipid level in hepatopancreas and increased lipid 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 [32,33]. Murugesan et al. [34] noticed eyestalk ablation influenced protein, lipid and carbohydrate content in ovarian maturation of crab, *Charybdis lucifera*. Varalakshmi and Reddy [35] found significant changes in carbohydrate, protein and energy leading to ovarian maturation in *Macrobrachium lanchesteri* due to eyestalk ablation. Decrease in lipid classes in the hepatopancreas and significant increase in ovarian tissue due to unilateral eyestalk ablation in fresh water female crabs, *Oziotelphusa senex senex* for ovarian maturation was reported by Samyappan et al. [19]. Guan et al. [36] 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 [21] studied the effect of eyestalk ablation in fresh water 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. [37] also noticed increased gonadosomatic indices and gonad maturation of freshwater crab, *Paratelphusa hydrodromous* due to unilateral eyestalk ablation. Ikhwanuddin Mhd. et al. [38] found increase in gonadosomatic index and mean oocyte diameter which indicated ovarian maturation due to eyestalk ablation of blue swimming crab, *Portunus pelagicus*.

#### 4. CONCLUSION

Thus, the findings of the present study correlate with the available literature. The lipid levels in the ovary seems to be elevated due to injection of exogenous  $\beta$ -

estradiol hormone followed by decreased lipid levels in the hepatopancreas. Moreover, eyestalk ablation showed significant increase in lipid levels in the ovary and decrease in lipid levels in the hepatopancreas over exogenous  $\beta$ - estradiol hormone injected crabs. The increase lipid levels in ovaries and decrease in lipid levels of hepatopancreas showed the ovarian maturation. Nonetheless, the study opens a platform for further investigation to confirm in detail the study that has been carried out by the author.

#### COMPETING INTERESTS

Author has declared that no competing interests exist.

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