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Effect of estradiol and 17 α -hydroxyprogesterone on ovarian development of fresh water paddy field crab *Ozotelphusa senex senex* (Fabricius)

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ABSTRACT

Reproductive physiology is highly controlled and governed by the variety of hormonal factors. The present study have been investigated the role of estradiol and 17 α - hydroxyprogesterone in the reproduction of the fresh water field crab *Ozotelphusa senex senex* regulated by the nervous and endocrine systems. The crabs were divided into two experimental groups and injected with estradiol and 17 α - hydroxyprogesterone @10 mol/crab separately at different intervals in one month duration of experiment. The Gonado Somatic Index (GSI), Oocyte Diameter (OD) and ovarian indices were estimated in both control and experimental female crabs. All the parameters were increased significantly ($P<0.01$) when compared with controls, the estradiol was more effective than 17 α -hydroxyprogesterone and both are influencing the gonadal growth and reproduction in female crab *Ozotelphusa senex senex*. Possible impact of steroids on reproduction of crustaceans is discussed.

Keywords: Estradiol , 17 α - hydroxyprogesterone, Ovarine development, *Ozotelphusa senex senex*.

INTRODUCTION

Decapod crustaceans represent a large, diverse biological group with significant potential as an aquaculture resource. Large scale penaeid shrimp culture industries currently exist in Asia and Central America. During the past two decades our understanding of crustacean reproductive endocrinology, especially that of the female, has grown steadily. Reproductive physiology in crustaceans is highly controlled and regulated by the nervous and endocrine systems (Engelmann, 1994). Endocrine control of female reproduction is governed by a variety of hormonal and neuronal factors that involve neuropeptide hormones, such as gonad stimulating hormone (GSH) and vitellogenesis-inhibiting hormone (VIH), like ketosteroids such as ecdysteroids; and sex steroids such as estradiol and progesterone (Huberman, 2000; Zapata *et al.*, 2003). Ecdysteroids are the primary hormonal factors of molting and positively affect vitellogenesis also (Subramoniam, 2000). Vertebrate-type steroids have been reported to be present in the hepatopancreas, ovary, and hemolymph of crustaceans, their levels changing in correlation with the oocyte maturation cycle (Lafont and Mathieu, 2007). The main objective of the present study is to evaluate the influence of steroid hormones on ovarian growth of the fresh water field crab *Ozotelphusa senex senex*. Throughout the investigation the effect of estradiol and 17 α -hydroxyprogesterone has been studied separately and simultaneously.

MATERIALS AND METHODS

The injections were given through the arthroal membrane of the coxa of the walking legs by using hypodermic syringe one time in a week for four weeks duration of four doses. Throughout the study period, the injections were given at the beginning of every week and at the end of the week, some animals were sacrificed for experimental analysis. No deaths are observed in the control or in the experimental group throughout the study period.

Gonad somatic index (GSI):

The gonadal index was calculated at weekly intervals throughout the study period. Initially, the whole animal weight is recorded. Then the gonads (usually 'H' Shaped) were isolated completely and weighed. The gonado somatic index was calculated by applying the following formula of Pillay and Nair (1971). The weights of the animal and the gonads were recorded in an electrical balance.

$$\text{Gonadal index} = \frac{\text{Wet weight of gonad}}{\text{Wet weight of animal}} \times 100$$

Oocyte Diameter (OD):

After 24 hours of fixation in aqueous Bouin fluid, the ovaries were dehydrated through an alcohol series cleared in xylene and then embedded in paraffin (Melting point 56-58°C). Sections of 7 µm thick were cut and stained with hematoxylin and counterstained with eosin. The diameters of 50 randomly chosen oocytes from each ovary were measured using an ocular micrometer on compound microscope.

RESULTS AND DISCUSSION

The process of reproduction in a species is influenced by a series of endogenous and various exogenous factors. The gonadal index has been considered as a tool to know the maturation. Reproductive physiology in crustaceans is highly controlled and regulated by the nervous and endocrine systems (Engelmann, 1994). Endocrine control of female reproduction is governed by a variety of hormonal and neuronal factors that involve neuropeptide hormones, such as gonad-stimulating hormone (GSH) and vitellogenesis inhibiting hormone (VIH).

Results obtained on ovarian index (OI), and oocyte diameter (OD), of control and steroids (estradiol and 17α-hydroxyprogesterone) injected female field crab *Oziotelphusa senex senex* at different time intervals (1st, 2nd, 3rd and 4th week) are presented in tables 1 and 2 and also the percent changes of ovarian index (OI) and oocyte diameter (OD) are presented in figures 1 and 2.

Mean values of ovarian index (OI) recorded at different time intervals of control and estradiol and 17α-hydroxyprogesterone injected female field crab *Oziotelphusa senex senex* at different time intervals are presented in table 1 and corresponding percent changes in figure 1. It is clear from the results that the ovarian index (OI) was significantly (P<0.01) higher in estradiol and 17α-hydroxyprogesterone injected *O. senex senex* than the controls at different time intervals. It is also clear that the magnitude of increase in ovarian index (OI) was much greater in *O. senex senex* injected with estradiol and 17α-hydroxyprogesterone. Amongst the steroids, estradiol injected crabs showed greater percent increment in ovarian index (OI) compared to 17α-hydroxyprogesterone injected crabs.

Mean values of oocyte diameter (OD) of ova in female field crab *O. senex senex* was recorded from control and steroids (estradiol and 17α-hydroxyprogesterone) injected experimental groups. Values presented in table 2 and the corresponding percent changes in figure 2. It is evident from the results that the oocyte diameter (OD) was significantly (P<0.01) higher in estradiol and 17α-hydroxyprogesterone injected crabs than the controls. It is also clear that the magnitude of increase in oocyte diameter was much greater in *O. senex senex* injected with estradiol compared to 17α-hydroxyprogesterone injected.

Stimulation of ovarian growth is clearly observed in crab *Oziotelphusa senex senex* after injection of estradiol and 17α-hydroxyprogesterone hormones. This was evidenced by a significant increase in ovarian index (OI) in estradiol injected female crabs followed by 17α-hydroxyprogesterone when compared to controls. Similar results have been observed in red swamp crayfish *Procambarus clarkii* (Rodriguez *et al.*, 2002b). Reddy *et al.*, (2006) demonstrated that these hormones induced ovarian growth and ovarian vitellogenin synthesis in *O. senex senex*. This study shows that injection of estradiol and 17α-hydroxyprogesterone increase in vitellogenin in early-vitellogenic females.

Further the works of S.R. Warriar *et al.*, (2001) Ramachandra Reddy *et al.*, (2006) and E.Coccia *et al.*, (2010) showed similar results reflecting increased ovarian index (OI) in the *O. senex senex*, cray fish *Cherax albidus* and mud crab *Scylla serrata* respectively. Several studies viz, progesterone, pregnenolone, 17 α -hydroxyprogesterone, 6 β -hydroxyprogesterone, 20 α -hydroxyprogesterone, 17 β -estradiol, etc., have been identified in inducing ovarian growth in different crustaceans (Fingerman *et al.*,1993, Subramoniam, 2000;Tsukimura, 2001; Wilder *et al.*, 2002; Oetken *et al.*, 2004). It is well known that the circulating steroid hormones induce oocyte growth in oviparous vertebrates. A significant positive correlation of circulatory estradiol- 17 β and progesterone with ovarian development was reported in the giant tiger shrimp *Penaeus monodon* (Quinito *et al.*, 1994).

Fig: 1; Percent change in ovarian index (OI) in response to estradiol and 17 α -hydroxyprogesterone injected female rice field crab *Oziotelphusa senex senex*

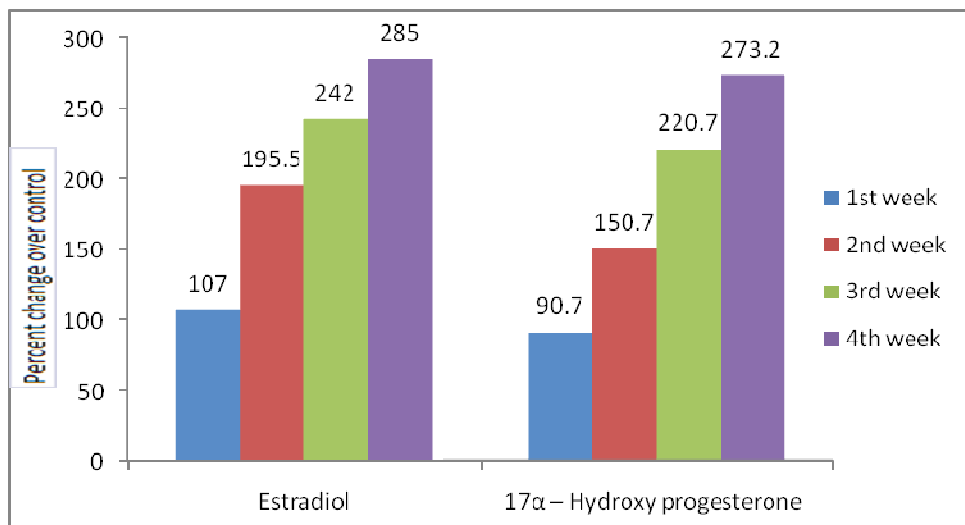


Fig: 2; Percent change in Oocyte Diameter (OD) in response to estradiol and 17 α -hydroxyprogesterone injected female rice field crab *Oziotelphusa senex senex*

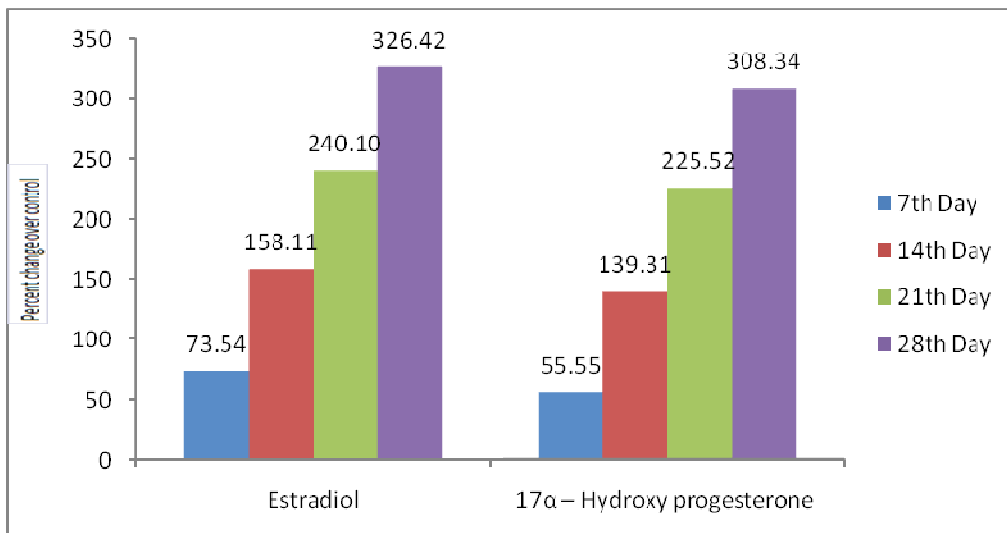


Table 1; Mean values (Mean \pm SD) of Change in Ovarian Index (OI) in control and estradiol and 17 α hydroxyprogesterone injected individuals of female field crab *Oziotephusa senex senex*

Experimental Groups	Control	Estradiol injected	17 α - hydroxyprogesterone injected
1 st week	0.065 \pm 0.002	0.135 \pm 0.009	0.124 \pm 0.013
2 nd week	0.067 \pm 0.008	0.198 \pm 0.013	0.168 \pm 0.007
3 rd week	0.069 \pm 0.011	0.236 \pm 0.065	0.221 \pm 0.042
4 th week	0.071 \pm 0.014	0.274 \pm 0.082	0.265 \pm 0.063

Table – 2; Mean values (Mean \pm SD) of Change in Oocyte Diameter (OD) in control and estradiol & 17 α -hydroxyprogesterone injected individuals of female field crab *Oziotephusa senex senex*.

Experimental Groups	Control	Estradiol injected	17 α - hydroxyprogesterone injected
1 st week	18.9 \pm 0.34	32.8 \pm 0.45	29.4 \pm 0.12
2 nd week	19.1 \pm 0.19	49.3 \pm 0.14	45.71 \pm 0.65
3 rd week	19.2 \pm 0.09	65.80 \pm 0.53	62.5 \pm 0.09
4 th week	19.3 \pm 0.22	82.3 \pm 0.68	78.81 \pm 0.68

CONCLUSION

In several crustaceans, increased oocyte diameter and change in colour of ovary were used as criteria to determine ovarian maturation (Chariniaux- Cotton and Payen, 1988). The ovarian maturation was also determined by considering the concentration of vitellogenin in hemolymph and accumulation of yolk globules in oocytes (Tsukimura, 2001). The oocyte diameter (OD) was significantly higher in steroid injected crabs when compared with controls. This clearly indicates that the administration of steroid hormones increased oocyte diameter thereby enhancing the ovarian growth. The results are in agreement with the observations of Ramachandra Reddy *et al.*, 2006; Subramoniam, 2000; E.Coccia *et al* 2010; Kishori *et al.*, 2001; Gosh and Ray, 1994 in different crustacean species. The results of the present study clearly demonstrated that the estradiol and 17 α -hydroxyprogesterone have significantly improved the ovarian index (OI) and oocyte diameter (OD), of fresh water field crab *Oziothephusa senex senex*.

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