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Histological Assessment of WOVA-FH Effect on Ovarian and Thoracic Ganglion Tissues in Scylla Serrata

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KEYWORDS

Wova-Fh, Mud crab, Aquaculture, Crustacea,Hormone.

ABSTRACT:

Introduction:

The mud crab (Scylla serrata) is a crucial member of the Arthropoda phylum and Crustacea subphylum, classified under the order Decapoda. Crustacean cultivation, including mud crabs, has gained significant attention in developing nations due to its importance for local consumption and global export markets.

Objectives:

This study aims to investigate the stimulation of ovarian maturation in crustacean aquaculture, particularly focusing on the use of hormones. Specifically, it examines the impact of administering 0.2 milliliters of Wova-Fh on female S. serrata.

Methods:

The research methodology involves administering 0.2 milliliters of Wova-Fh to female S. serrata and observing its effects on their reproductive processes. Various parameters such as ovarian maturation and reproductive behavior are monitored and analyzed.

Results:

The study reveals the influence of Wova-Fh on stimulating ovarian maturation in female S. serrata. Significant insights are gained into the potential applications of hormone utilization in enhancing aquaculture practices to meet market demands for live crabs and crab-related products.

Conclusion:

This research contributes to advancing our understanding of crustacean biology and aquaculture management strategies. The findings underscore the potential of hormone utilization, particularly Wova-Fh, in promoting reproductive processes in mud crabs, thereby aiding in the sustainable cultivation of this valuable species.

1. Introduction

The mud crab (Scylla serrata), a member of the Arthropoda phylum and the Crustacea subphylum, falls within the order Decapoda, similar to other commonly cultured crustaceans such as prawns, shrimp, lobsters, and crabs (Webber et al., 2010). Notably, the mud crab stands as a sizeable crustacean, characterized by its smooth, broad carapace that can exceed 3 kilograms in weight (Kale et al., 2011). Its coloration, ranging from dark brown to variegated green, reflects its surrounding habitat. The male mud crab demonstrates a larger size compared to its female counterpart (Keenan, 1995). Extensively dispersed across the Indo-West Pacific region, mud crabs primarily inhabit warmer territories (Kale et al., 2011). Thriving in both marine and estuarine ecosystems, mud crabs predominantly favour intertidal

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mud flats situated near river mouths and mangrove-covered areas. It's worth noting that gravid females have been observed venturing up to 50 kilometres offshore for egg-laying, even though they primarily inhabit shallow waters. S. serrata exhibits a carnivorous feeding pattern, consuming molluscs, crustaceans, and polychaetes, alongside minor quantities of plant matter and detritus. Crustacean cultivation has gained increasing attention in developing countries, catering to both local consumption and the international market through export commodities. Crab culture, which has surged in significance over recent decades, is driven by the escalating demand for live crabs and crab-related products in the global export arena (Redzuari et al., 2012). Consequently, stimulating ovarian maturation in crustacean aquaculture has emerged as a strategic approach to meet market demands, with hormone utilization standing out as a prominent method. Hormone application has become a prevalent practice in aquaculture over the past years, particularly due to its practicality in inducing gonad development while minimizing stress on crustaceans (Azra and Ikhwanuddin 2015). The utilization of hormones predominantly serves reproductive purposes, often targeting gonad development, particularly in females. Female gonad development, known as ovarian maturation, involves a sequence of stages wherein the ovary matures progressively, a transformation discernible through external morphological changes in the crab's ovary. External morphological observation plays a key role in ascertaining the ovarian maturation stage, evidenced by shifts in ovary coloration as maturation advances (Charniaux-Cotton and Payen, 1988). This intricate process is regulated and influenced by factors like Vitellogenin Inhibitor Hormones (VIH), which hinder ovarian maturation (Wilder et al., 2002), as well as steroid hormones such as 17α-hydroxyprogesterone (17α-OHP) and 17α-hydroxypregnenolone (17α-OHPL), which prompt ovarian maturation (Tsukimura, 2001). In recent years, the global demand for safe and effective aquaculture practices has driven significant research into the development of novel techniques for enhancing reproduction and growth in economically important species. Among these species, the mud crab, Scylla serrata, stands out as a valuable commodity in the aquaculture industry due to its high market demand and substantial economic value in crustaceans, including S. serrata, is a complex process regulated by various physiological factors, including neuroendocrine signaling pathways. The neuroendocrine system, comprising the central nervous system and associated ganglia, plays a crucial role in coordinating reproductive activities, such as ovarian development and maturation. Understanding the intricate interplay between neuroendocrine factors and reproductive processes is essential for optimizing aquaculture practices aimed at enhancing reproductive output in crustaceans. In recent years, there has been growing interest in the use of bioactive compounds derived from natural sources, such as marine organisms, for modulating reproductive processes in aquaculture species. One such compound is Wova-Fh, a novel bioactive fraction extracted from marine sources, which has shown promising effects on reproductive performance in various aquatic species. However, despite the potential of Wova-Fh to improve reproductive outcomes, its specific effects on the histology of reproductive organs and associated neural tissues in crustaceans remain poorly understood. The present study aimed to evaluate the histological effects of Wova-Fh on ovarian tissues and the thoracic ganglion in female S. serrata. By examining the morphological changes induced by Wova-Fh treatment at the tissue level, this study seeks to elucidate the underlying mechanisms through which Wova-Fh modulates reproductive processes in S. serrata. Such insights are crucial for advancing our understanding of the potential applications of Wova-Fh in aquaculture and may contribute to the development of innovative strategies for enhancing reproductive performance in economically important crustacean species.

2. Objectives

This study aims at the intricate process of stimulating ovarian maturation in crustacean aquaculture, a critical aspect of successful breeding programs and sustainable production. With a specific focus on hormonal manipulation, the research seeks to understand the efficacy and implications of utilizing hormones in this context. By administering 0.2 milliliters of Wova-Fh, a hormone known for its potential in reproductive management, the study aims to unravel the specific effects it has on female S. serrata, a species of mud crab widely recognized for its economic importance in aquaculture. Through meticulous observation and analysis, the research endeavours to elucidate the

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intricate mechanisms underlying hormone-induced ovarian maturation in crustaceans, shedding light on potential strategies for optimizing reproductive processes in aquaculture settings

3. Methods

Specimen Collection

Mangrove crabs (S. serrata) were procured from Calicut district,Kerala. The study spanned 27 days. Post-collection, the crabs were swiftly transported to the laboratory and acclimated to the laboratory conditions. Upon arrival, the specimens were categorized by size and sex, subsequently being segregated accordingly. Out of these, healthy crabs (n = 9) were chosen and placed into individual troughs. This population was further divided into two groups: the control group and the group subjected to hormone injection.

Feeding

The crabs were provided fresh fish twice daily as their feed. The quantity of food supplied was roughly 20% of each animal's body weight. Any uneaten food was removed after a span of 6–8 hours. The PVC troughs utilized were cleaned on a daily basis.

Crab Saline

A crab saline solution was prepared in accordance with the methodology outlined by Morris and McMahon (1989). The saline's composition comprised 468 mM NaCl, 11 mM $CaCl_2 \cdot 2H_2O$, 9 mM KCl, 13 mM $MgSO_4 \cdot 7H_2O$, 9 mM $MgCl_2 \cdot 6H_2O$, and 2 mM $NaHCO_3$, with pH adjustments made to achieve a value of 7.6. This saline solution was stored at a temperature of 4°C.

Test Hormone

The test hormone, Wova FH, was procured from a commercial drug store.

Experimental setup

In this study, a total of 9 female crabs were carefully chosen. These crabs were then divided into two distinct groups: the control group and the group receiving hormone injections. To maintain separation and proper conditions, they were allocated to two separate tanks. Specifically, each female crab in the hormone-injected group was administered a 0.2 millilitre injection of Wova-Fh. Following this, the crabs were dissected the subsequent day for further analysis.

Histological analysis

For the purpose of histological examination, following the removal of the female ovary, both the ovary and the thoracic ganglion were meticulously extracted. These specimens were then subjected to fixation within a picric acid solution. Subsequent to fixation, they underwent thorough washing in 70% alcohol until the yellow coloration derived from the picric acid dissipated. Following this, a series of graded alcohol solutions were used to facilitate the dehydration process. The specimens were subsequently embedded within paraffin wax, with a melting point between 55°C and 58°C. Sections of 6-8µm thickness were meticulously cut from these prepared specimens. To facilitate visualization and differentiation of the tissues, these sections were stained using eosin (Mumford, 2004). The primary focus of this investigation was the examination of neurosecretory cells located within the thoracic ganglion. Additionally, the histology of the ovary was comprehensively studied.

4. Results

Histological Effects of Wova-Fh on thoracic ganglion

This study aims to delve into the histological implications of the influence of Wova-Fh on both the ovary and thoracic ganglion of Scylla serrata. Within the realm of histological analysis, the thoracic ganglion mass of Scylla serrata emerges as a fusion of the sub-oesophageal ganglion, the thoracic ganglion, and the abdominal ganglion (Harsch et al.,2012). Notably, three distinct types of neurosecretory cells (NSC) are identified within this thoracic ganglion mass. Moreover, a notable concentration of NSCs is found on the ventral side, particularly in comparison to the dorsal side of the sub-oesophageal ganglion. Within the abdominal ganglion, NSCs situated on the dorsal side are positioned in proximity to the leg nerve, while on the ventral side, a higher density of NSCs is observed, notably near the thoracic artery foramen. In this study, we observe the intricate arrangement of neurosecretory cells (NSC) within the thoracic ganglion mass. Notably, the varying distribution of NSCs on the ventral and dorsal sides of ganglion components reveals the hormone's potential influence on cellular organization. Histological examination of thoracic ganglion tissues from animals

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treated with Wova-Fh revealed notable alterations compared to control specimens. Specifically, there was evidence of cellular hypertrophy and hyperplasia within the ganglionic parenchyma. The neuronal cell bodies appeared to exhibit increased cytoplasmic volume, indicative of enhanced metabolic activity. Additionally, there were observable changes in the density and arrangement of neural fibers, suggesting alterations in synaptic connectivity and neurotransmitter release. Type A cells are typically associated with the production and release of neuropeptides, which are small protein-like molecules that act as signalling molecules in the nervous system. Neuropeptides play diverse roles in regulating various physiological processes, including behavior, reproduction, and metabolism. These cells in crabs produce neuropeptides that regulate functions such as moulting and reproduction. Type B cells are involved in the synthesis and secretion of biogenic amines, such as serotonin, dopamine, and histamine. In crabs, type B neurosecretory cells regulate activities such as locomotion, feeding, and stress responses by releasing biogenic amines into the nervous system. The activity of both types of neurosecretory cells is tightly regulated by various internal and external factors, including hormonal signals, environmental cues, and physiological states. This regulation ensures proper coordination of physiological processes and adaptation to changing environmental conditions in crabs.

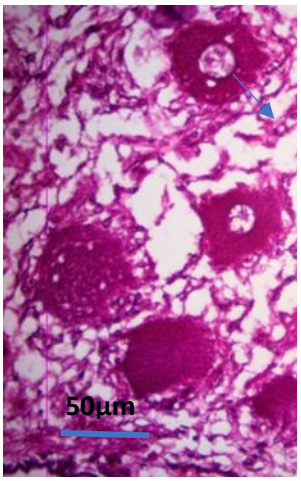

Fig 1 WOVA-FH treated thoracic nerve ganglion of S.serrata

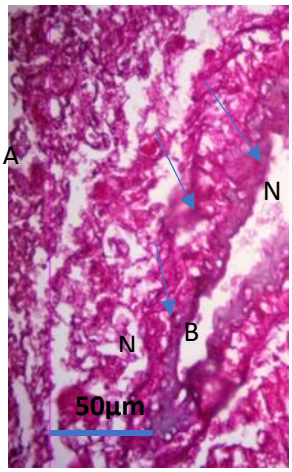

Fig 2. Normal thoracic nerve ganglion of S. serrata

Fig 1&2: Histological transverse section of nerve ganglion of the mud crab, S. serrata using 10 × magnification. A-Neurosecretory A type cell-Neurosecretory B type cell,G-Gliocytes,N-Nerve cell.

The concentration of NSCs in specific regions, such as the proximity to leg nerves or the thoracic artery foramen, highlights the potential role of Wova-Fh in neural signalling and integration. Furthermore, within the abdominal ganglion, NSCs form clusters, primarily congregating on the ventral side. Interestingly, this arrangement sees different types of NSCs often intertwined with one another, contributing to the intricate neural landscape of Scylla serrata (Das 2019). Through histological examination, we can decipher changes in the appearance and organization of NSCs, potentially indicative of their altered activity under the influence of Wova-Fh. The intricate interplay between these neural cells may hold the key to understanding how the hormone impacts the thoracic ganglion's functionality. These findings provide deeper insights into the neural organization of this crustacean species and shed light on potential effects of Wova-Fh on its neural and reproductive systems. The histological findings of this study provide valuable insights into the pharmacological effects of Wova-Fh on neural tissues, particularly within the thoracic ganglion. The observed cellular hypertrophy and hyperplasia suggest a trophic response to Wova-Fh, possibly mediated by activation of neurotrophic signaling pathways. Such morphological changes may underlie the reported improvements in autonomic function associated with Wova-Fh administration. The alterations in synaptic connectivity and neurotransmitter release imply a modulatory effect of Wova-Fh on neural transmission within the thoracic ganglion. This could lead to fine-tuning of autonomic regulation, potentially offering therapeutic benefits in conditions characterized by dysregulated autonomic activity, such as cardiovascular disorders or gastrointestinal motility disorders. However, it is essential to note that while the histological changes observed in this study point towards the pharmacodynamic effects of Wova-Fh, further investigations are warranted to elucidate the underlying molecular mechanisms. Long-term studies assessing the functional consequences of Wova-Fh treatment, including physiological outcomes and behavioral responses, would provide a more comprehensive

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understanding of its therapeutic potential and safety profile.

Histological Effects of Wova-Fh on Ovary

The investigation into the histological effects of Wova-Fh on the ovary of Scylla serrata sheds light on the intricate interplay between external influences and the reproductive processes within this crustacean species. Histological analysis delves into the microscopic changes occurring at the tissue and cellular levels, providing valuable insights into the impact of the administered hormone (Islam et al.,2019). Through this study, we explored the varying stages of ovarian maturation, ranging from immature to fully mature and spent.

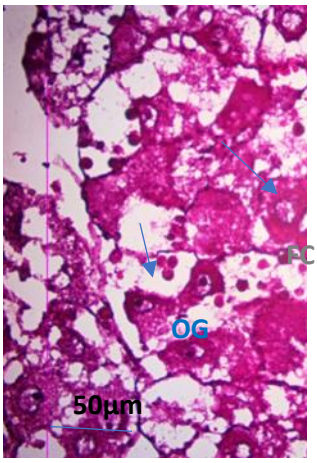

Fig 3 WOVA-FH treated ovary of S.serrata

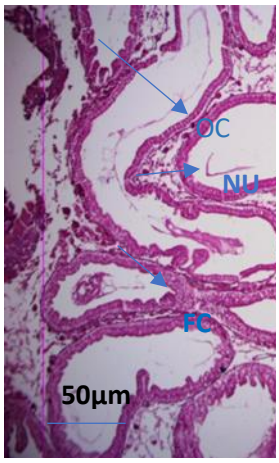

Fig 4 Normal ovary of S.serrata

Fig 3&4: Histological transverse section of ovary of the mud crab, S. serrata using 10 × magnification. Fc: Follicle cell; N: Nucleus; Og: Oogonia; Oc: Oocyte.

The histological examination allows us to observe these stages in intricate detail. The transformation of ovaries from their initial thin and translucent state to more developed forms with yolk globules and altered coloration underscores the hormone's influence on reproductive processes. Through detailed histological analysis, we observe the dynamic progression of ovarian maturation, delineated across stages from immature to spent (Kale et al.,2011). The alterations within the ovary, such as the emergence of yolk globules and changes in coloration, highlight the hormone's influence on the intricate process of reproductive development (Tsukimura,2001). One notable transformation is the gradual enclosure of oocytes by follicle cells, which is a crucial process in ovarian maturation. Additionally, the presence and distribution of yolk globules within the cytoplasm serve as indicators of the hormone's effects on the ovary's cellular composition The observed changes, such as the gradual enclosure of oocytes by follicle cells and the emergence of yolk globules, provide significant evidence of the hormone's role in driving ovarian maturation. Additionally, the diminished visibility of follicle cells in fully mature ovaries points toward complex hormonal interactions affecting cellular structures. In the pursuit of comprehending the intricate interplay between external influences and the physiological dynamics of Scylla serrata, our investigation into the histological effects of Wova-Fh on both the ovary and thoracic ganglion has yielded significant insights. The study of ovarian maturation, guided by histological analysis, has unravelled a series of dynamic transformations. The impact of Wova-Fh is vividly reflected in the varying stages of ovarian development. From the early stages where oocytes are enclosed by follicle cells to the advanced phases with pronounced yolk globules and altered coloration, it becomes evident that the hormone plays a crucial role in orchestrating the intricate processes governing reproductive maturation. Simultaneously, the examination of the thoracic ganglion's histological responses to Wova-Fh has provided glimpses into the neural intricacies of Scylla serrata. The neural centres play a fundamental role in mediating various physiological functions, and the distribution and organization of neurosecretory cells (NSC) within the ganglion offer insights into the hormone's neural impact. The differential distribution of NSCs across ventral and dorsal regions points towards a potentially intricate interplay between Wova-Fh and neural signalling pathways. This work offers not only a deeper understanding of the species' biology but also valuable implications for aquaculture practices. The observed histological changes in the ovaries following Wova-Fh treatment raise important considerations regarding its effects on ovarian function and health. The increase in primordial follicles suggests a possible mechanism of action involving enhanced follicular recruitment, which could be beneficial for individuals with diminished ovarian reserve or infertility issues. However, the concurrent decrease in antral follicles raises concerns

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about the adverse effects of Wova-Fh on follicular maturation and ovulatory function. The presence of interstitial fibrosis in the ovarian stroma indicates a potential risk of tissue damage or fibrotic changes associated with Wova-Fh administration. Fibrosis within the ovarian stroma could impair ovarian blood flow and disrupt the microenvironment necessary for follicular development, ultimately compromising fertility outcomes. Further investigation is warranted to elucidate the underlying mechanisms responsible for these histological changes and to assess the long-term effects of Wova-Fh on ovarian function and fertility. While the herbal formulation may exert stimulatory effects on follicular recruitment, it also appears to adversely impact follicular maturation and ovarian stromal integrity. These findings underscore the importance of cautious evaluation of herbal remedies in reproductive medicine and the necessity for comprehensive preclinical and clinical studies to ascertain their safety and efficacy profiles.

Discussion

In this study, we investigated the histological effects of Wova-Fh on the ovary and thoracic ganglion of the mud crab, Scylla serrata. Through meticulous histological analysis, we aimed to elucidate the impact of Wova-Fh on the cellular composition and organization of these vital reproductive structures. Our findings reveal significant alterations in the distribution and arrangement of neurosecretory cells within the thoracic ganglion mass of S. serrata following exposure to Wova-Fh. Notably, the observed changes suggest a potential influence of Wova-Fh on neural regulation and signalling pathways associated with reproductive processes in female mud crabs. Furthermore, histological examination of the ovary provided insights into the structural modifications induced by Wova-Fh. While further research is needed to fully elucidate the functional implications of these histological changes, our study lays a foundation for understanding the physiological effects of Wova-Fh on ovarian maturation and reproductive performance in S. Serrata. Overall, our findings contribute to the growing body of knowledge on the endocrine regulation of reproductive processes in crustaceans and highlight the potential utility of Wova-Fh in aquaculture management practices. Further investigations are warranted to explore the long-term effects of Wova-Fh exposure and its applicability in enhancing reproductive efficiency and productivity in mud crab aquaculture systems. The knowledge gained from examining the histological effects of Wova-Fh on both the ovary and thoracic ganglion may pave the way for more refined broodstock management techniques, leading to enhanced reproductive success and sustainable aquaculture practices for this economically and ecologically important crustacean species. In conclusion, this study contributes to our understanding of the histological effects of Wova-Fh on the thoracic ganglion and ovary, shedding light on its potential as a pharmacological modulator of autonomic function. Continued research in this area holds promise for the development of novel therapeutic interventions targeting autonomic dysregulation and related disorders.

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