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STRUCTURE OF VASA DEFERENTIA AND SPERMATOPHORE IN *METAPENAEUS MONOCEROS* (DECAPODA: PENAEIDAE)

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ABSTRACT

The morphology of male reproductive system of *Metapenaeus monoceros*, an important commercial species is described. The testicular lobes are connected though thread like tubules which joins the vasa deferentia. The proximal vasa deferentia are thinner than median vasa deferentia which show a whorl and its inner hard material create septum whereas each terminal ampulla has single mature spematophore.

Key words: Spermatophore, vasa derentia, *Metapenaeus monoceros.*

INTRODUCTION

Family Penaeidae has immense commercial value. Among 25 species of shrimps in Pakistani waters, only 12 species has been commercially exploited (Kazmi, 2003; Majid, 1988). On the basis of size and color *Metapenaeus monoceros* and *Metapenaeus affinis* are locally categorized as Kalri group of shrimps (Ayub and Ahmed 1991). Both of the species are more or less equally abundant.

Male reproductive organs of penaeid prawn have received lesser attention. The work on structure of male reproductive organs and formation of spermatophores has been undertaken mostly in species of the genus *Penaeus*: (King, 1948; Bauer and Min, 1993; Medina, 1995; Jeri, 1998). Work done from Pakistan on this aspect is restricted to Tirmizi and Khan (1970), Huq (1973, 1981) Sultana (1985), Sultana *et al.* (1994). To the best of our knowledge no study has been undertaken on morphology of reproductive system and its spermatophore formation in *Metapanaeus monoceros*.

MATERIAL AND METHODS

The samples of *Metapenaeus monoceros* were collected from commercial fish landing sites along Sindh Coast during September 2007 to July 2008; the collected samples were transported to the laboratory in insulated boxes under ice. Shrimps were stored at -40 ^{0}C until dissected. For histological studies, samples were fixed with 10% neutral formalin, dehydrated with isopropanol, and embedded in paraffin; 5-7 µm sections were obtained and standard H&E staining protocols were followed. Spermatophores were obtained by pressing the ejaculatory duct. A sum of 60 specimens were dissected for morphological studies.

RESULTS

Fig. 1a presents the freshly dissected male reproductive system.

Testicular Lobes (TL)

Testes are five to seven finger-like projections of unequal length. The testicular lobes reveal highly convoluted and minute seminiferous tubules through the transparent membrane (Fig.1a & b). Each testicular lobe opens into a thin tubule which runs along the axial edges of the testicular lobes (Fig.1b). The testicular lobes are connected with each other through this tubule. The same tubule connects to the Proximal Vasa Differetia (PVD) laterally (Fig.1b). In immature specimens the testicular lobes were translucent. The testicular lobes continuously pour the spermatic content into the PVD, that's the reason two or three testicular lobes were found partially empty and flattened, whereas remaining swollen and completely filled.

Proximal Vasa Deferentia (PVD)

It is relatively thin, long, loosely coiled and tubular structure which is transparent, when there is no spermatic mass inside and looks opaque when gelatinous spermatic mass is present; some irregular cells were also seen.

Medial Vasa Deferentia (MVD)

The MVD is relatively broader in diameter than both PVD and DVD. The diameter is not uniform throughout its length it is broad in the median part. The MVD form a single loop or whorl by twisting along its median length (Fig.1c). The spermatic material was almost observed inside the tube, which rendered this portion an opaque appearance. It indicates that spermatic material is processed here for quite some time and the individual cells here tend to agglomerate to form small clumps of spermatozoa. The dense mass of non-spermatic material is also distinguishable as a milky white sheet like structure and is distinguishable (Fig.1c),

Distal Vasa Deferentia (DVD)

The DVD is the narrowest and straight part of vas deferens, small clumps of spermatic cells were found in this region which passed on to TA for further processing to form a composite spermatophore.

Terminal Ampulla (TA)

The TA is an oval structure attached to the end of DVD (Fig.1d). It is located at the bases of the fifth pair of pereiopods and can be visually observed as a bulging white structure. The terminal ampulla is milky white and its opening is located laterally on the opposing side of distal vas deferens, which serves to ejaculate the spermatophore. The opening has a small flap. The upper part of TA has a median thick muscular fold, which serve as a partition for spermatic and non spermatic materials. The upper part of TA lodges the hard white non-spermatic pad like material (Fig.1E). On the lower side of TA, there is a separate sac bearing spermatic material, where the final development of spermatophores takes place.

Spermatophore

Fig1F reveals the shape and structure of the spermatophore*;* the front portion consists of a soft and gelatinous aggregate mass of spermatozoa, whereas end portion has hard non spermatic hard material forming a plug like structure. In all of the specimens studied, each terminal ampulla contains two spermatophores at the same time, one is fully developed and other is under development.

Formation of Spermatophores

The observation of spermatic mass from different parts of the vasa deferentia and TA has given some clue to the formation of spermatophores. The spermatic mass from the testicular lobes enter into the proximal vasa deferentia; at this stage spermatozoa are irregular in shape and of different sizes, whereas in MVD, they assume a more regular, almost rounded and of uniform size. MVD is visibly divided into two parts for separate transportation of spermatic and non-spermatic materials (Fig. 5). Both type of materials transported through DVD to the TA.

In the terminal ampulla, processing of both types of materials evolve a composite spermatophores. The non spermatic material forms a gelatinous sheet which has been folded over itself to form a bivalve like appearance; this folded structure positioned itself in a way that as soon as the fully formed and agglomerated spermatozoa entered to the TA, deposited inside the median cavity between the folded parts. A plug-like structure is also formed by the non-spermatic hard material first as a separate structure , later it is loosely attached to the folded sheath bearing spermatozoa and it assumes the final shape of spermatophore. The complete spermatophore finally move towards the opening and ejaculated. The studies show the highest degree of maturity of spermatozoa, where it forms a sperm cell takes place within the terminal ampulla. The sperm cells are rounded disc shaped and non motile.

Histology

Testicular lobes

Each testicular lobe is composed of very minute seminiferous tubules, which are convoluted and forms a dense mass. These seminiferous tubules are actually the site for processing of male reproductive cells. An apparently double layer membrane forms the wall of each tubule; the outer membrane is termed as tunica or membrane propia (in homology with the membrane in *Penaeus setiferus* (as described by King, 1948) and inner layer of epithelial cells, which is supposed to be germinative in function. The sperm cells are released to the vasa deferentia as spermatid, (Fig. 2A) where they further increase in size and became more regular in shape.

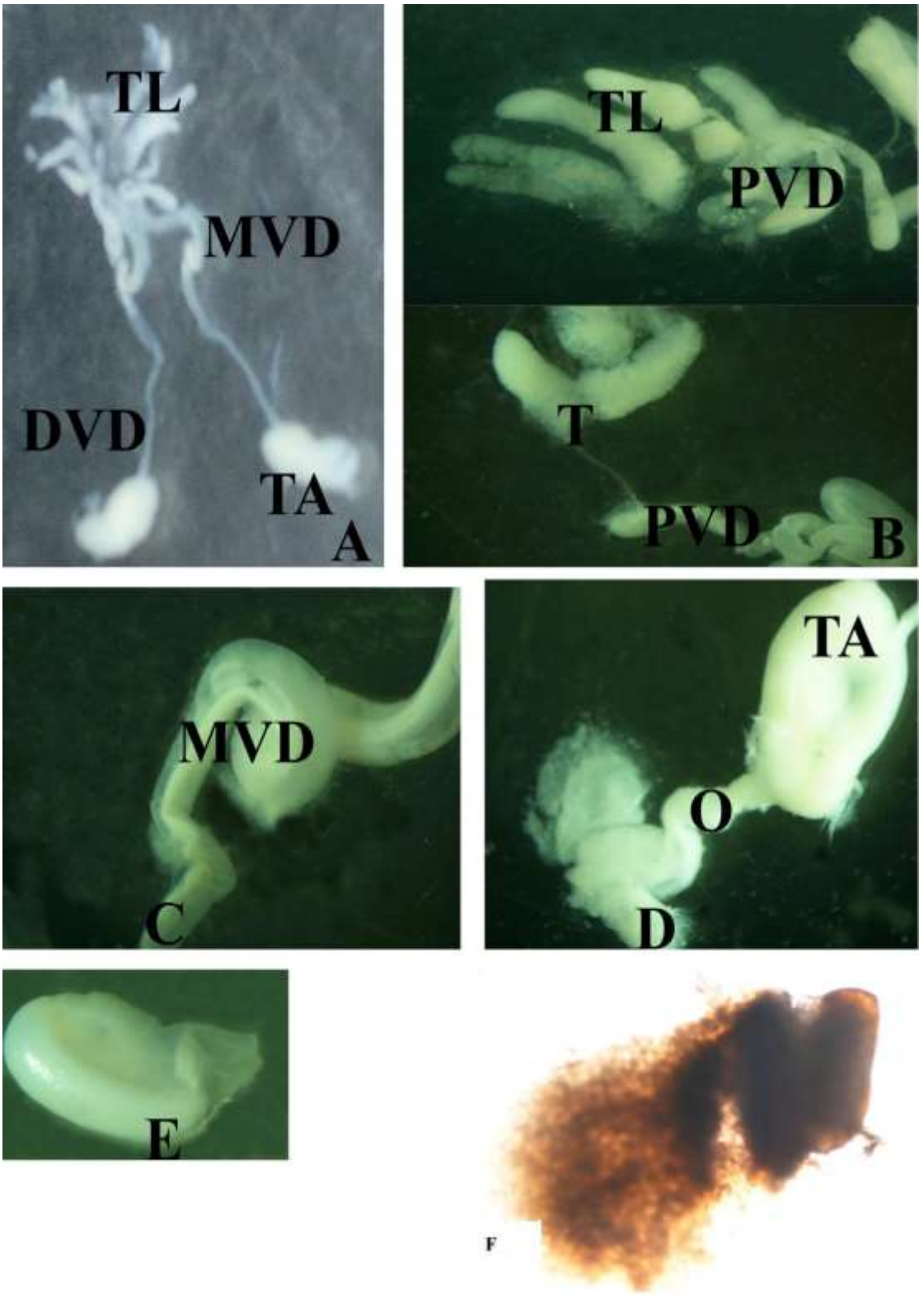

Fig. 1. A. Showing the complete reproductive system. B. Showing testicular lobes, proximal vasa deferentia, and tissue which connected with proximal vasa deferentia. C. Showing the median vasa deferentia wholf. D. Showing the Terminal ampulla. E. Showing the hard part. F. Showing the spermatophore. DVD. Distal vasa deferentia; MVD. Median Vasa deferentia, O. Opening; PVD. Proximal vasa deferentia. T. Thin tissue, TA. Terminal ampulla

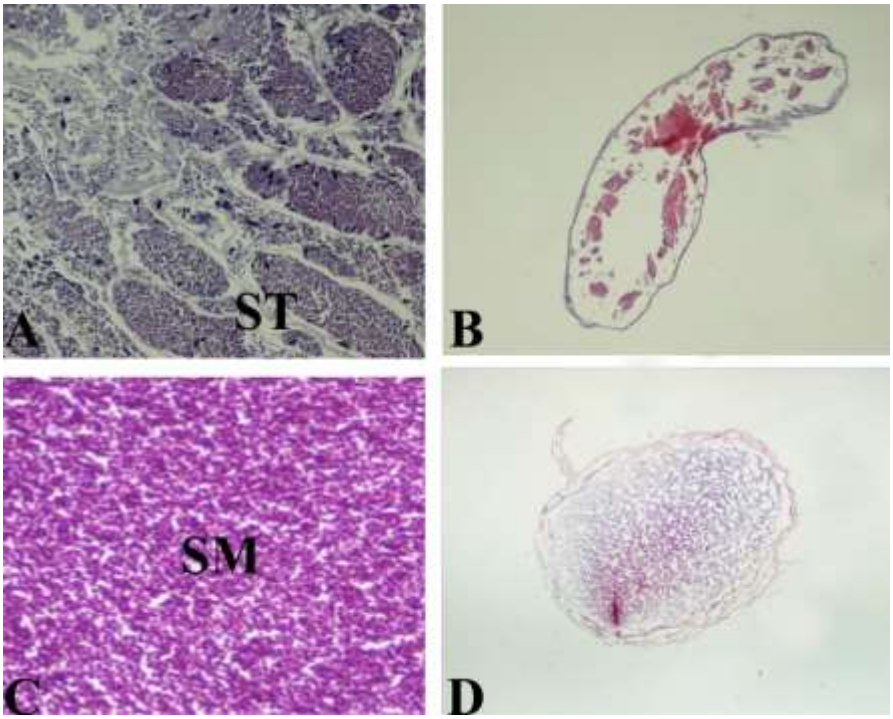

Fig. 2. A. Showing the transverse section of testicular lobe having seminiferious tubules, B. Showing the section of proximal vasa deferentia having no septum. C.median vasa deferentia , showing sperm mass. D. Transverse section of terminal ampulla. SM. Sperm mass.

Proximal Vasa Deferentia

It is only a tube like structure, no septum or partitioning was seen the lumen is somewhat irregular in shape it has a slight curve which makes the tube somewhat narrow in right end and broad in the left end , the structure is surrounded by thin layer of epithelial cells Fig. 2B.

Medial Vasa Deferentia

This region of vasa deferentia has two ducts namely spermatic duct and accessory duct. The epithelia of spermatic ducts are accessory ducts separated by connective tissue. The curved part after the whorl between the anterior and posterior parts of median vasa differentia roughly coincided with detachment of one end of the septum; accessory layer began to flow into the spermatic duct. The spermatic mass obsereved in median vasa deferentia (Fig. 2C)

Distal Vasa Deferentia

There is some internal partitioning, which is incomplete, it is attached from one side to the wall of DVD but the other side is detached. The margins of septum not straight due to its hanging position in the duct.

Terminal ampulla

The terminal ampulla has a muscular wall (Fig. 2D). The main components of the ejaculatory duct are the squamous epithelium under line with the connective tissue, several bands of circular muscles then a wide zone which appears to be composed of interspersed longitudinal and circular muscle fiber, another layer of connective tissue and finally the thick glandular epithelium cells. The spermatic sac is connected with the net work of connective tissue; the sac is surrounded by epithelial cells. The spermatic part is extended into the posterior part of TA and non spermatic part is lodged by anterior part only.

DISCUSSION

The present work deals with the structure of vasa deferentia, spermatophores and of penaeid shrimp, *Metapenaeus monoceros*. The structure of spermatophores and vasa deferentia has been thoroughly studied in *Penaeus* spp, (Malek and Bawab, 1974 a & b; Perez Farfante, 1975; Champion, 1987; Ro *et al*., 1990; Bauer and

Cash 1991; Chow *et al*., 1991) and *Trachypenaeus similis* (Bauer and Min, 1993).The basic division of the vasa deferentia in *Metapenaeus monocers* has followed the same pattern found in Penaeus species that is visible in four parts; viz, proximal vasa deferentia (PVD), medial vasa deferentia (MVD), distal vasa deferentia (DVD) and terminal ampulla (TA). In *Metapenaeus monoceros* testicular lobes were elongated and distinctly separated finger like projections. The proximal vasa deferens is a loosely coiled long tube. The medial vasa deferens (MVD) is broader in diameter than both PVD and DVD and turned around itself to form a single whorl at its middle length. The terminal ampulla is invariably oval and large; the opening to ejaculate the spermatophores is lateral and covered by a flap. In *Metapenaeus monoceros* only one large, mature spermatophore was found in each terminal ampulla i.e. a single pair is released at a time. An accessory plug like non spermatic material is attached to the spermatic mass as a part of spermatophore, which may probably used to push the soft gelatinous agglomerated mass of sperm cells into the female thelycum and then it is lost or detached, since it was not found attached to the thelycum of any female in specimen. The various parts of vasa deferentia are modified along its length to perform various functions of producing the outer layers to cover the loose mass of spermatozoa and also the accessory structures which facilitate the sperm transfer and storage after insemination. This has been elaborated in detail in *Penaeus setiferus, P. aztecus,* and *P. kerathurus*, (Malek and Bawab, 1974 a&b. Ro *et al*., 1990; Bauer and Cash 1991; Chow *et al*., 1991; Baur and Min, 1993).The histological work shows that each testicular lobe is composed of very minute seminiferous tubules, which are convoluted and forms a dense mass. The seminiferous tubules contained various developmental stages of sperm cells. The sperm cells are released to vasa deferentia as spermatozoa, where they further increase in size and became more regular in shape. The proximal vasa deferentia have no septum or partitioning where as median vasa deferentia has two ducts namely spermatic duct and accessory duct. The epithelia of spermatic ducts are accessory ducts separated by connective tissue, in distal vasa deferentia internal has partitioning, which is incomplete, the septum is attached from one side to the wall of DVD but the other side is detached. The margin of septum not straight due to its hanging position in the duct.The terminal ampulla has a thick muscular wall. Spermatozoa are in spermatic sac; the sac is surrounded by epithelial cells. The anterior part of terminal ampulla shows the partitioning of spermatic and non-spermatic parts, whereas posterior or terminal part bears only the spermatic part, which actually represent the position of spermatophore, as the spermatic part is extended into the posterior part of TA and non spermatic part is lodged by anterior part only.

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