

## HISTOLOGICAL OBSERVATIONS ON DEVELOPMENT OF SPERMS IN *LITOPENAEUS VANNAMEI* (BOONE, 1931) (CRUSTACEA:DECAPODA)

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### ABSTRACT

We conducted the histological observations on development of sperms in white leg shrimp *L. vannamei*, a prime aquaculture species. The testes have three distinctive stages of sperms, spermatogonium, spermatocytes and spermatids, in addition the nurse cells. The spermatozoa were observed in distal vas deferens (DVD) and in ejaculatory duct. The spike of the spermatozoa is prominent. We concluded that development of the sperms is a continuous process all along the reproductive tract.

**Key words:** Histology, genesis, sperms, reproductive tract.

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### INTRODUCTION

*Litopenaeus vannamei* Boone, 1931), an economically important shrimp was introduced from Hawaii, America to China in 1988, and technique of artificial cultured larvae was made successful in 1992. Up to now, over 1000 hatcheries are in operation in China and 300000 tons and 210000 tons harvested during 2003 in marine and freshwater farming, respectively (Qing and Hai, 2005).

The development and structure of sperms in Crustacea remains an interesting subject and several valuable publications have appeared recently on *Penaeus chinensis* (Wu *et al.* 2001), *Solenocera membranacea*, *S. africana*, *Pleoticus muelleri* (Medina *et al.* 2006) *Ucides cordatus* (Castilho *et al.* 2007), *Armases rubripes* (Santos *et al.* 2009). Diaz *et al.* (2002) stated that male reproduction is valuable for productivity of brookstock of captive penaeids and it is very hard to find the maturity stages of male species by visual observations (Abraham *et al.* 2007). Several histological observations on the structure of male reproduction system in penaeid shrimps have been reported, such as *Penaeus setiferus* (King, 1948) *Penaeus indicus* (Subrahmanyam, 1965; Mohamed and Diwan, 1993), *Metapenaeus dobsoni* (Vasudevappa, 1992), *Metapenaeus monoceros* (Abraham, *et al.* 2007). In *L. vannamei* and ultrastructural studies of sperms in testis of *L. vannamei* also been also have published in chinese ( Xu *et al.* 2010).

The present investigation has focused on the developmental pattern of sperms along the whole reproductive tract in *L. vannamei* by means of histological methods. Our aim was to collect a basic data on spermatogenesis of *L. vannamei*.

### MATERIALS AND METHODS

The samples of *L. vannamei* used in the present study were obtained from a shrimp hatchery Zhanjiang, China in 2009. Tissues were fixed in Bouin's solution for 24 h, washed thrice with 70% ethanol, and stored at 4 °C until use. The tissues were serially dehydrated in a ascending ethanol series (85%, 95%, 100%) and cleared with xylene, and then embedded in paraffin wax. The serial transverse and longitudinal sections were cut at 6-7 µm on rotary microtome. Slides were stained by haematoxylin and eosin (H&E) and examined and photographed with Nikon E80i microscope.

### RESULTS

The seminiferous tubules are convoluted and the development of the sperms takes place within the seminiferous tubules. The results revealed that three types of cells are present in seminiferous tubules of testis, the spermatogonia, spermatocytes and spermatids, respectively, in addition the nurse or nutritive cells are also distinct (Fig 1 A- B). The presence of spermatozoa in distal vas deferens (DVD) suggested the continuation of sperm development within the reproductive tract ( Fig 1 C), at this division the sperms are showing spike (Fig. 1D). The compact mass of mature sperms are visible in ejaculatory duct (Fig.1E), where as the spike in most of the spermatozoa are clearly visible (Fig.1F). In *L. vannamei* all the mature sperms (spermatozoa) are ejaculated by means of hemispermaphore.

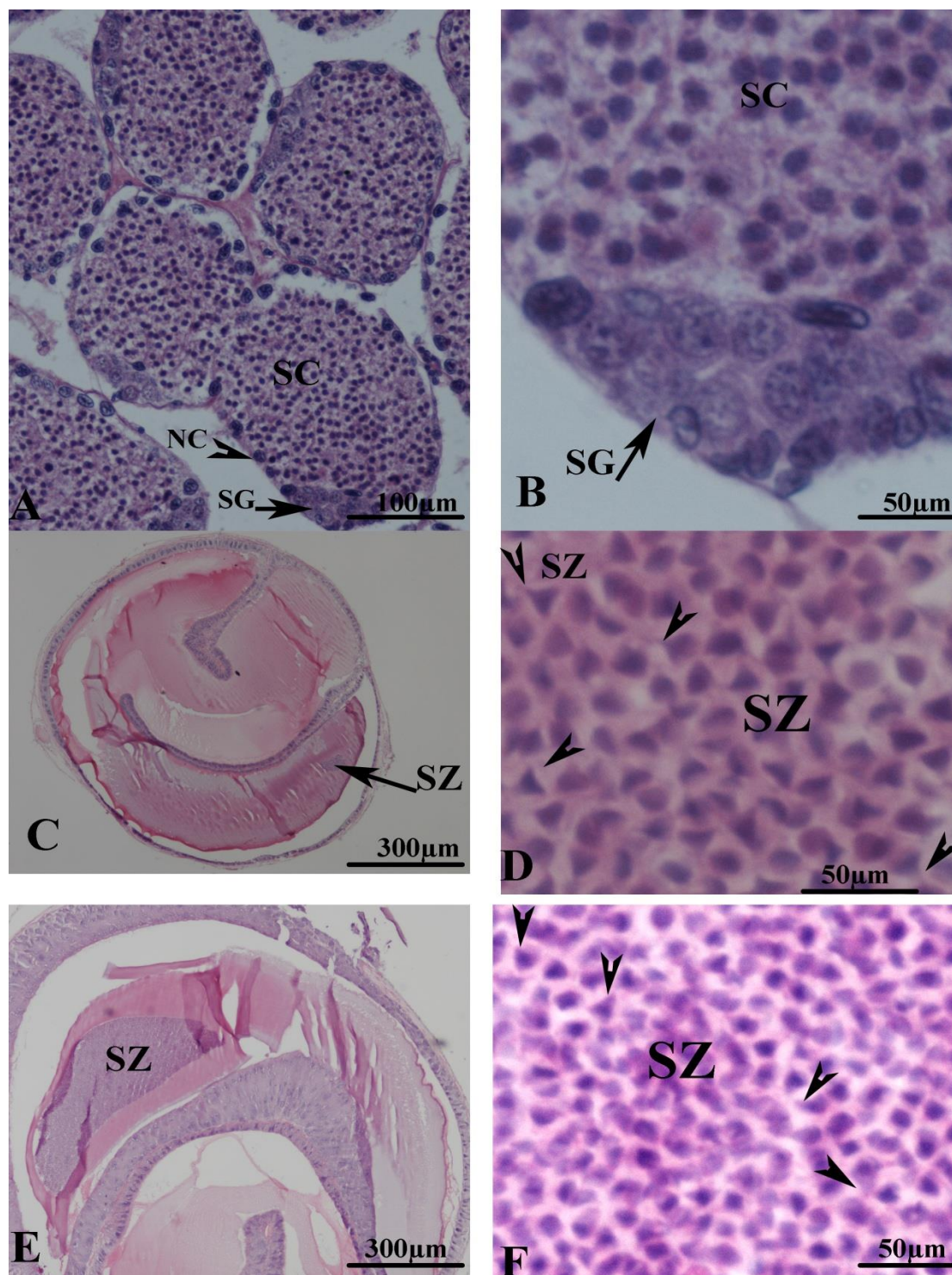


Fig. 1. A. Transverse section of testis showing seminiferous tubules, spermatogonia, spermatocytes and nurse cells or nutritive cells. B. Higher magnification of testis, showing spermatogonia and spermatocytes. C. Transverse section of distal vas deferens showing the spermatozoa. D. Higher magnification of distal vas deferens showing the spermatozoa with spike. E. Transverse section of ejaculatory duct showing spermatozoa. F. Higher magnification of spermatozoa showing spike.

NC, Nurse cells; SC, Spermatocytes; SG, Spermatogonia; SZ, Spermatozoa; Arrow head shows spike.

## DISCUSSION

The histology of testis and vasa deferentia have been studied and described in this study. Earlier, several histological and ultrastructural reports have been published for spermatogenesis. In *Metapenaeus monoceros* (Abraham *et al.*, 2007) described five stages in testis. Similarly (Xu *et al.*, 2010) described five stages in *L. vannamei* although we did not see the spermatozoa in testis of *L.vannamei*, however, the spermatozoa with its spike are prominent in distal vas deferens and ejaculatory duct. Our results are in agreement with the studies of Wu *et al.* (2001) who stated that sperms develop in testis and mature in vas deferens. In *M. monoceros*, Muhammad (2009) also described maturity of sperm in vas deferens. The results suggest that development of sperms in *L. vannamei* are in synchronous pattern. We conclude that important aspect of spermatogenesis for aquaculture are needed to be observed for whole life of the adult male. Parnes *et al.* ( 2006 ) have demonstrated the cyclic disposal of old spermatophores, timed by the molt cycle, in a marine shrimp, *L. vannamei*.

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