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INDUCTION OF GAMETE ACTIVITY IN THE INDIAN PENAEID PRAWNS

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In fish and other vertebrates sperms are motile and motility is an easy assay for determining viability of sperms but in crustaceans it is not so. Here induction of activity in nonmotile spermatozoa denoted by acrosomal filament formation is considered as a prime factor for assessing the viability. Similarly, fertile eggs can be assessed by inducting activity in them and denoting certain characteristics. Such basic information is most essential for developing cryopreservation techniques.

Introduction

Penaeid shrimps are very important crustaceans in several respects. They are commercially important, making bulk of the catch in shrimp fisheries around the world. In recent years they have become a focus of aquaculture/ mariculture industry. In evolutionary order they are unique among Decapoda in a sense that they have primitive method of reproduction in which fertilized embryos are not brooded but spawned free into the water hatching as nauplii. Yet inspite of their commercial ecological and phylogenetic significance, many aspects of reproduction in such animals are not known properly.

In recent times, due to awareness of cryogenic preservation of fertile gametes to enhance animal production, extensive studies are being made on sperm-egg characteristics of several commercially important species. In decapod crustaceans particularly on marine shrimps such studies are meagre. In penaeid prawns the sperms are reported to be always non-motile and non-flagellated. Earlier one or two reports mention about the motile sperms in penaeids but these studies were based on simple light microscopy. Later attempts made by many with Transmission and Scanning Electron Microscopy revealed more structural details and nature of the sperm. For example George Brown in 1966 and G. W. Hinsch in 1971 described the ultrastructure of the sperm of few marine crabs. Similarly, detailed molecular structure of the sperms of the prawn *Palaemon* sp. has been given by L.D. Koehler in 1971 and Papathanassiu and King in 1984 and that of Lobster by Talbot and his associates in 1980 and W.J. Dougherty and his co-workers in 1986. Lynn and Clark in 1983 have done SEM studies on sperm-egg interaction in the freshwater prawn *Macrobrachium rosenbergii* whereas in penaeid prawn *Sicyonia ingentis* Wallis Clark and his associates have carried out indepth investigation on sperm-egg activational changes through ultrastructural means. In penaeids, though several other aspects of reproduction have been studied, not much has been done on activational changes of gametes and their interaction. Recently CMFRI has conducted studies on induction of activity in gametes of *P. indicus* and *P. monodon* and achieved some interesting results. In fact in fish and other vertebrates sperms are motile and motility is an easy assay for determining viability of sperms but in arthropod crustaceans it is not so. Here induction of activity in non-motile spermatozoa denoted by acrosomal filament formation is considered as a prime factor for assessing the viability. Similarly fertile eggs can be assessed by inducting activity in them and denoting certain characteristics.

Induction of activity in spermatozoa

The morphological and ultrastructural features of non-motile (unreacted) spermatozoa in case of Indian white prawn (*P. indicus*) and tiger prawn (*P. monodon*) studied in the Institute's laboratory revealed that superficially the sperms are distinguished into three regions viz. 1. a posterior main body, 2. a central cap region and 3. an anterior spike. The posterior main body is an elongate sphere housing an uncondensed nucleus followed by the central cap region which includes acrosomal vesicles. The nucleus is not membrane bound and extremely fibrillar. The prominent membrane bound vesicles are commonly seen near the margin of the cell body. In the cap region two portions can be distinguished by differences in electron density. Towards the anterior side a distinct spike is seen. The ultrastructure of the spike shows that it contains an amorphous electron dense material with some cross-striations in between.

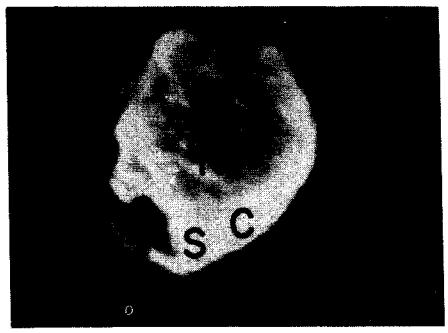

Fig. 1. Electronmicrographs of the spermatozoan.
A. Scanning Electron Microtophoto (SEM) of the sperm of *Penaeus monodon* x 2500.
B. Transmission Electron Microtophoto (TEM) of the sperm of *P. monodon* x 8000.
(A-acrosome; C- cap; N-nuclceus; S- spike and V-vesicle).

Literally though the penaeid sperms appear to be non-motile they become active just prior to fertilization. The activated or reacted sperms exhibit a total change in their morphological anatomy. It has been found that in the wild when sperm contact an egg at the time of spawning, it binds with the tip of the spike to the egg's vitelline envelope and in no time gets activated. The first manifestation of activated sperms in *P. indicus* and *P. monodon* as observed in our laboratory is the loss of anterior spike which immediately results in the externalization of the acrosomal vesicle contents and exocytosis of the acrosomal vesicles. With the exocytosis of the acrosomal vesicle sperm activation is completed by forming a long acrosomal filament.

In recent years Wallis Clark and others of Bodega Marine Laboratory in California have done extensive studies and described the detailed events of physiological control and mechanism of sperm-egg activation, fertilization and early development in penaeid prawn *Sicyonia ingentis*. In fact it is reported that non-motile sperms of decapod crustacea are richly diverse group in terms of both morphology and types of activational changes that precede fertilization. For example un-reacted reptantian sperm possesses numerous appendages that emanate from the nucleus. During acrosome reaction sperm undergo "eversion" process resulting by forming an amorphous material. On the other hand sperms of caridean natantian has been described as unistellate, possessing only one appendage and do not undergo an acrosome reaction as a prerequisite to fertilization. But the sperms of penaeid natantian though they are unistellate, undergo acrosome reaction during sperm-egg interaction.

What is known to be the acrosome reaction, has been varidely described earlier as sperm "explosion" or sperm "eversion" or "degeneration". Barker and Austin in 1963 correctly identified the explosive phenomenon as an event analogous to the acrosome reaction of flagellated sperm. The acrosome reaction which was carefully studied in CMFRI laboratory for penaeids actually involved cell eversion process i.e., sperm is everted inside out. The fine structural events of acrosome reaction which occur during sperm-egg interaction have been described by Brown in 1966 for blue crab, *Callinectes sapidus*. While numerous studies of sperm development and mature sperm structure exist in literature, knowledge about the acrosome reaction of the decapod sperm is comparatively scarce. Talbot and Chanmanon in 1980 have mentioned that acrosomal reaction of the sperm is a must and considered for generating the forward movement of this otherwise immotile spermatozoa.

In recent times it was discovered that the acrosomal reaction can be artificially induced by using solution hypotonic to seawater. Few workers have activated the acrosomal reaction in the laboratory by using either egg-water or divalent ionophores. In *P. indicus* and *P. monodon* induction of sperm activation was carried out in CMFRI laboratory by using egg-water and also by ionophores like bromocalcium and velinomycin (SIGMA). Activation time could be improved by proportionately increasing the concentration of activating agents. Egg-water was collected from freshly spawned eggs. To

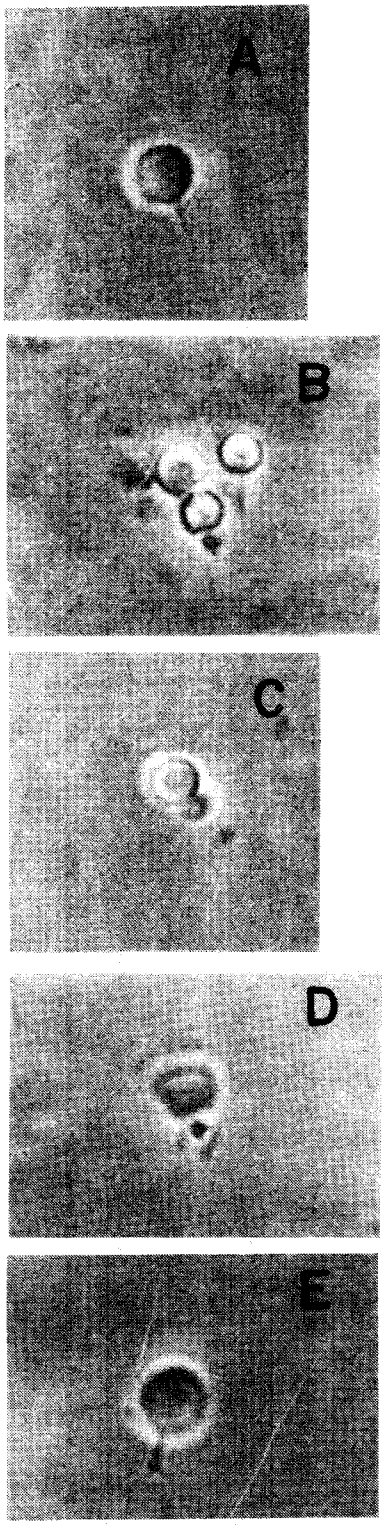

Fig. 2. Phase micrographs of the five activational states of *Penaeus indicus* sperm x 400. (A) an unreacted sperm possessing an anterior spike; (B) a sperm that has undergone acrosomal exocytosis and has lost the spike; (C) initiation of acrosomal filament formation; (D) acrosomal filament formation in progress and (E) a sperm that has completed the acrosome reaction by forming an acrosomal filament.

collect egg-water, a female *P. indicus* is spawned in a beaker containing 500 ml of seawater (S 30‰). After spawning the spawner was removed and the eggs were allowed to settle down. The whole volume of water was slowly reduced to one third by decanting and the eggs were then gently swirled in remaining water for 10-15 minutes. After allowing the eggs to settle down the supernatant fluid (egg-water) was centrifuged and later it was frozen and stored in liquid nitrogen until needed.

Induction of egg activity

Information pertaining to the egg activation among penaeids is very much limited. Wallis Clark and his team have done detailed studies on egg activation in penaeid prawns viz., *P. japonicus*, *P. azetecus* and *S. ingentis*. In fact egg activation means such events as release of eggs from meiotic arrest immediately after the ovulation process, formation of hatching envelope around newly created zygote and switching on bio-synthetic machinery necessary for embryonic developement. It is described that fertilization is not the requisite to bring out activational changes in eggs. Even the eggs can be activated upon contact with seawater at the time of spawning. Therefore, the egg viability assay similar to that of sperm viability, becomes an important tool in cryopreservation technology.

CMFRI, has also done work on egg activation of *P. indicus* and some fascinating observations have been recorded. For egg activation studies the following procedure was adapted. Live spawner prawns were collected from wild and transported to the laboratory. After carefully assessing the riped maturity phase of the spawners they were dissected out to remove the eggs and the spermatophores separately. The eggs and sperms were then transferred to a 500 ml beaker containing 200 ml artificial seawater (S 35‰). The whole water was swirled gently and intermittently for about 5 to 10 minutes. This process allowed activation and fertilization of eggs. Immediately after that, the activational changes occurred in the eggs have been recorded systematically using phase-contrast microscope. Generally inactive and freshly ovulated eggs lack an enveloping jelly layer but posses jelly precursor material within extra cellular crypts of eggs and a very thin vitelline envelope. Upon activation, first the jelly precursor is expelled out from the egg surface crypts, surrounds the egg, and is transferred into jelly layer. Later in the

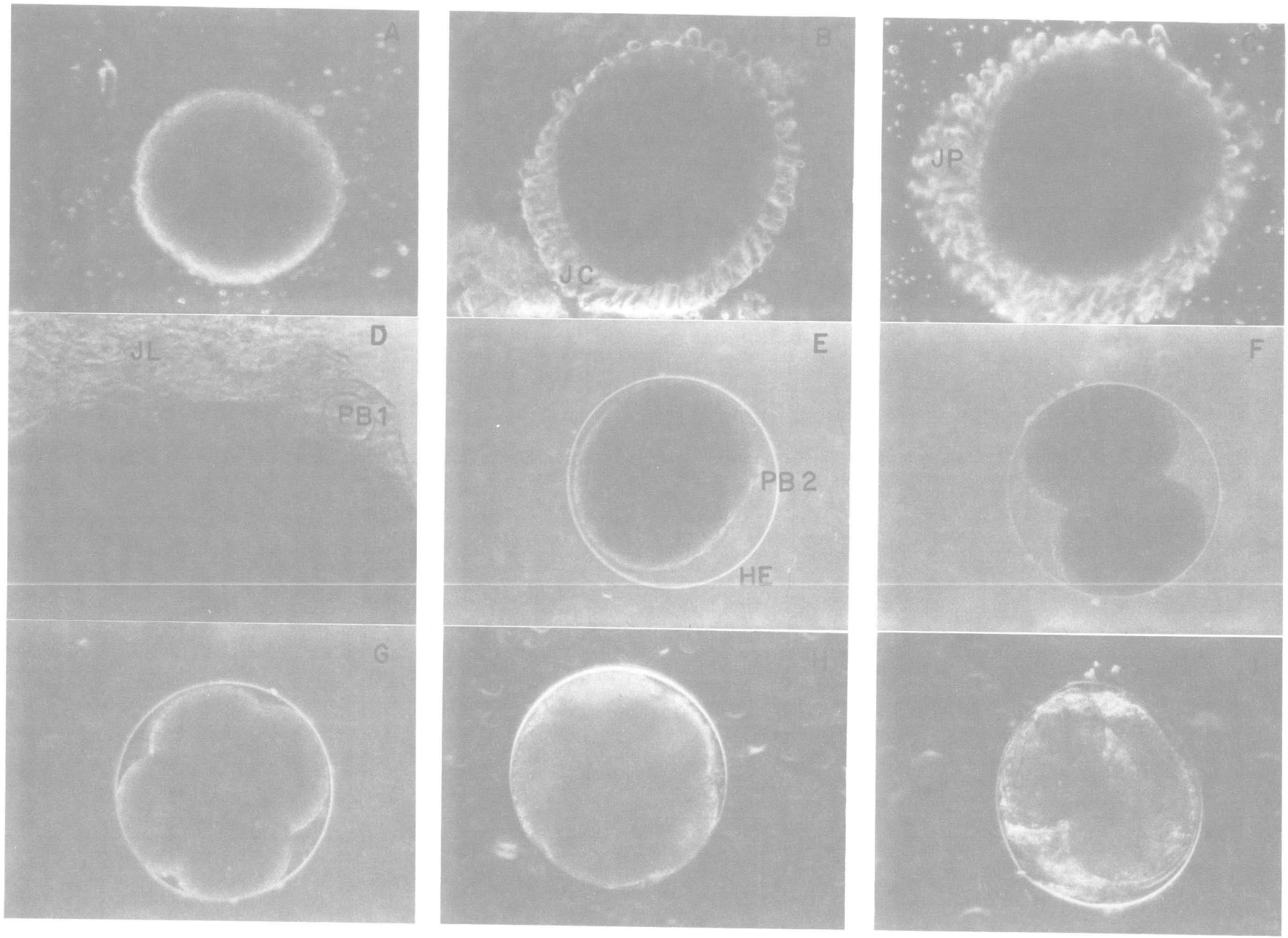

Fig. 3. Phase micrographs of the activational events in a fertilized egg of *P. indicus*. (A) Normal unactivated egg; (B) Activated egg containing jelly precursor in jelly crypts (JC); (C) Release of jelly precursor (JP) from egg upon exposure to seawater; (D) Jelly precursor is transformed into jelly layer (JL) with the release of first polar body (PB1); (E) Formation of hatching envelope (HE) is followed by release of the second polar body (PB2); (F) A normal first cleavage ensues; (G,H) successive cleavage of the egg and (I) Unfertilized egg undergoing an unequal abortive cleavage.

second step, egg resumes its first meiotic division by releasing the first polar body which is observed very distinctly in the activated eggs. This is again followed by the release of a second polar body after sometime. Finally the hatching envelope (protective extra cellular matrix) forms around the egg. Up to this stage of activation, fertilization process is not required, but the same is needed for the activated eggs to execute embryonic cleavages.