# The Acrosome Reaction of Sperm from *Paphia undulata*

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**ABSTRACT** Acrosome reaction (AR) of the sperm from *Paphia undulata*, the short-necked or baby clam, was induced with high calcium-artificial seawater (containing 0.3 M calcium chloride). Scanning electron microscopy revealed the occurrence of an average of 41.6 % ± 10.6 sd acrosome-reacted sperm upon the induction. The head of the acrosome-intact sperm consisted of a conical shaped acrosome (0.29 µm ± 0.02 sd long and 0.58 µm ± 0.05 sd diameter) and a conical shaped nucleus (1.29 µm ± 0.08 sd long and 1.28 µm ± 0.05 s.d. diameter). The AR was marked by the initial elongation of the anterior end of the acrosome followed by the rupture of the tip exposing an axial, rod-like, acrosomal process (AP). At the completion of the AR, the fully extended AP (0.51 µm ± 0.04 sd long and 0.16 µm ± 0.01 sd diameter) with a slightly wider base (0.28 µm ± 0.03 sd diameter) surrounded by the empty hull of the acrosome was formed.

KEYWORDS: acrosome reaction, sperm, *Paphia undulata,* clam.

## INTRODUCTION

*Paphia undulata*, the short-necked or baby clam, is one of the economically important bivalves of Thailand. They are widely consumed locally and are one of the food-product exports. However, harvesting is the only resource of the clams. The natural habitats are the mudflat areas along the east and the west coasts of the Gulf of Thailand. Tremendous decline of the natural populations currently is due to over-harvesting; therefore, increase production through farming may be a viable alternative.

Study of the reproduction and the fertilization of the clams is the prelude to a development of culture methods and farming. Although gonadal development and laboratory-rearing of the larvae have been described,[1,2] no other information concerning the reproduction of the clam has been reported. Mechanism of fertilization in the bivalves reported so far is similar to that of the other marine invertebrates. Occurrence of the acrosome reaction (AR) of the sperm is an essential stage prior to fertilization. The sperm undergo AR on the egg surface during fertilization. The AR consists of the fusion of the acrosomal membrane with the overlying plasma membrane, the release of a non-enzymatic protein (lysin) from the acrosome, and the generation of the acrosomal process (AP) from the anterior end of the exposed acrosome.[3,4,5,6,7] The AP is formed from the subacrosomal actin by polymerization of the monomeric actin or by forward movement of the preformed actin filament bundle.[8,9] Proteins coated on the surface of the AP mediate fusion of the surface of the AP with the plasma membrane of the egg.[10] The present study reports the induction of the AR of sperm from *Paphia undulata* by calcium, and the scanning electron microscopic observations of the sequential changes of the acrosome upon the AR.

## MATERIALS AND METHODS

Specimens of mature male *Paphia undulata* from Mae Klong, Samutsakorn Province, were purchased from the local markets. Gonads of the mature clams were rinsed and then lacerated in small volumn of the calcium-free artificial seawater (ASW). The ASW was composed of 423 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 23 mM $MgCl_2$, 25.5 mM $MgSO_4$ and 2.1 mM $NaHCO_3$. The released sperm were diluted in the calcium-free ASW and washed once by centrifugation at 600 g for 10 min. The sperm pellet was resuspended in the calcium-free ASW, or was induced to undergo AR by resuspending in the modified ASW containing high level of calcium chloride (0.3 M). The sperm were incubated for 30 min at room temperature and were then washed once in ASW. The washed sperm pellets were fixed in 10% (v/v) formalin in ASW, or in 4% glutaraldehyde in ASW for 1 hr at room temperature. The formalin-fixed samples were washed twice in ASW and were then prepared for light microscopy. The washed sperm suspensions were smeared, air-dried; and treated with Feulgen reagent. to stain the nucleus.

For staining of the acrosome, the air-dried specimens were treated with periodic acid-Schiff reagent followed by brief immersion in hematoxylin. The glutaraldehyde-fixed samples were washed in ASW and prepared for scanning electron microscopy. The sperm suspensions were dehydrated in a graded series of acetone and air-dried by dropping on small pieces of coverslip which were attached to the aluminium stubs. The specimens were then coated with gold palladium and examined under a scanning electron microscope. Beside observations of the acrosomal changes, numbers of the acrosome-reacted sperm in a total of 100 sperm of each specimen were scored in order to determine the percentage of the AR. Specimens from 3 replicate experiments were evaluated. Dimensions of the sperm head were determined from the scanning electron micrographs.

## Results and Disscusion

The nucleus of the *Paphia* sperm stained intensely with Feulgen reagent (Fig 1); whereas the acrosome stained negatively with Feulgen reagent (Fig 1) but positively with periodic acid-Schiff reagent (Fig 2). The acrosome was extremely small and hence was difficult to be visualized at the light microscopic level. Sperm counts from scanning electron microscopic observations indicated the induction of an average of 41.6% ± 10.6 sd acrosome reacted sperm by high calcium level. The head of the acrosome-intact sperm consisted of a conical shaped acrosome and a conical shaped nucleus (Fig 3). The acrosome was 0.29 µm ± 0.02 sd in length and 0.58 µm ± 0.05 sd in diameter (at the base of the acrosome). The nucleus was 1.29 µm ± 0.08 sd in length (from the base of the nucleus to the base of the acrosome) and 1.28 µm ± 0.05 sd diameter (at the widest portion). The midpiece comprised a whorl of 4 rounded mitochondria (Figs 3-6).

Variations from the AR reported in most bivalves was observed. Instead of the initial rupture of the anterior end of the acrosome followed by the elongation of the AP as observed in most bivalves, extension of the acrosomal tip apparently occurred prior to the disruption of the acrosome (Figs 4 and 5). Upon the rupture of the acrosome, an axial, rod-like, AP was exposed (Fig 5). At the completion of the AR, the fully extended AP was composed of 2 entities, a base of 0.28 µm ± 0.03 sd in diameter and a process proper of 0.51 µm ± 0.04 sd in length and 0.16 µm ± 0.01 sd in diameter (Fig 6).

Acrosome of the *Paphia* sperm is tiny in comparison with those of other bivalves reported. Acrosomes of the sperm from the abalone, *Haliotis discus,* and the mussel, *Mytilus edulis,* have been reported to be large or giant, and can be observed easily with light microscope.[6,11,12] Although acrosome of sperm from the oyster, *Crassostrea gigas*, is small, with a dimension of 0.3 µm in length and 0.8-0.9 µm in diameter,[13,14] it is about twice the size that of the sperm from *Paphia.* The length of the fully extended AP reported in various bivalves does not seem to correlate with the size of the acrosome. The AP of the sperm from *Haliotis* and *Mytilus,* which have large acrosomes, are 1 µm[12] and 5 µm[6] long, respectively. But the AP of the sperm from *Crassostrea*, which has small acrosome, is 11 µm[3] long. However, all the AP reported so far are explicitly longer than that from *Paphia.*

Slight variation from the general AR in the bivalves has been noticed in the AR of the sperm from *Crassostea.* Fusion of the acrosomal membrane with the overlying plasma membrane at the anterior tip of the acrosome followed by protrusion of the AP through the apex prior to the release of the acrosomal contents has been described.[14] Elongation of the acrosome prior to the rupture of the acrosomal membrane in the present observations seems to implicate an existence of a preformed internal structure. A preformed actin filament bundle has been described in the *Mytilus* sperm.[9] Initiation of the AR may trigger the forward movement of the preformed actin filament bundle and thus causes the elongation of the acrosome. Fusion of the acrosomal membrane with the overlying plasma membrane and generation of the AP, generally, occur sequentially but they involve independent mechanisms. Although the AP of the sperm from *Mytilus* is preformed, generation of the AP of most sperm involves polymerization of the monomeric actins[9]. Therefore, generation of the AP consumes longer time than membrane fusion in the AR of most sperm. But in the AR of the *Paphia* sperm, movement of a preformed structure is possibly induced primarily causes extension of the acrosomal tip prior to the commencement of the fusion of the acrosomal membrane with the overlying plasma membrane. This interesting feature of the AR in the *Paphia* sperm worths further detailed investigation.

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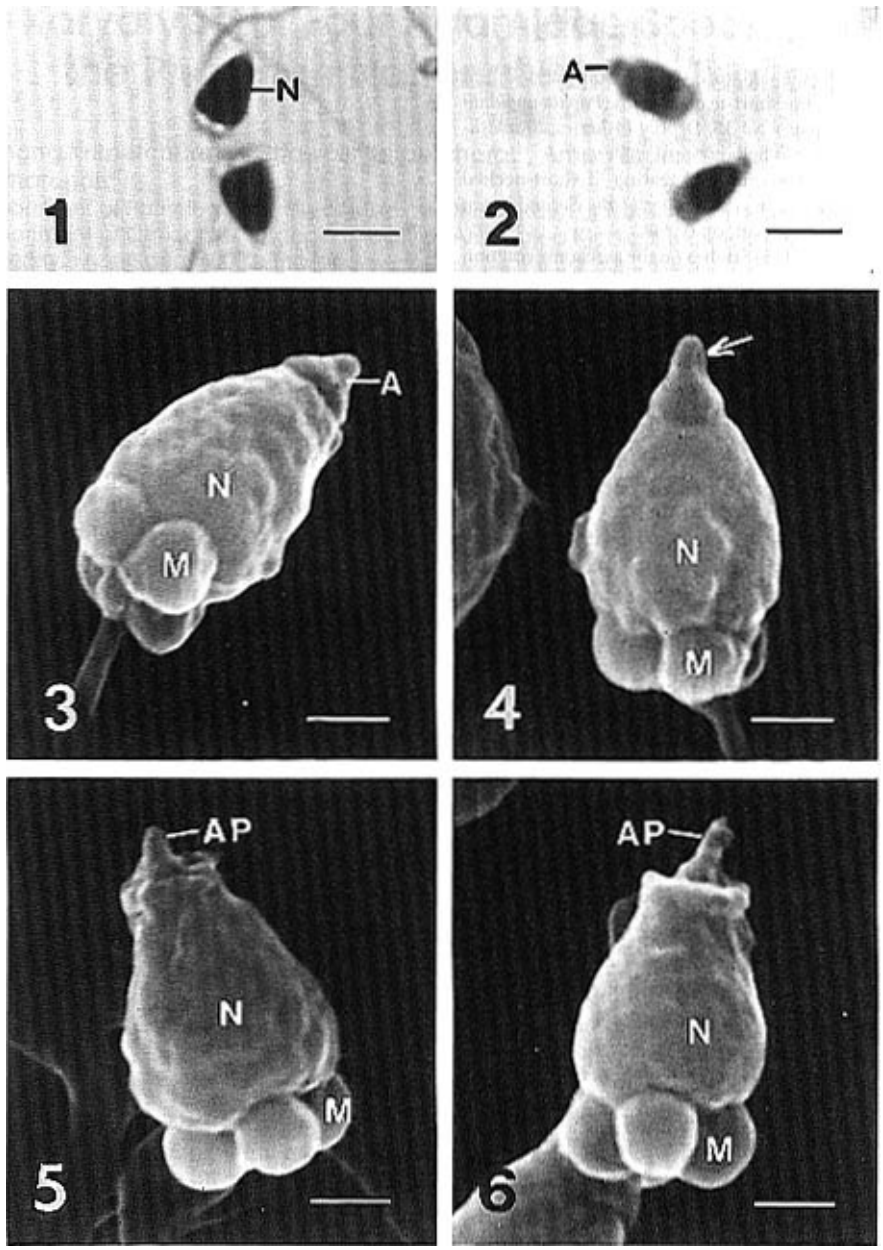


**Figs 1-2.** Light micrographs of the sperm from *Paphia Undulata.* Fig 1, Feulgen staining of the nucleus (N); Fig 2, periodic-acid Schiff-hematoxylin staining of the acrosome (A). Bars = 2 µm

**Figs 3-6.** Scanning electron micrographs of the sperm from *Paphia undulata* showing sequential changes of the acrosome during the AR. Fig 3, The acrosome-intact sperm contained a conical shaped acrosome (A), a conical shaped nucleus (N) and a whorl of 4 mitochondria (M); Fig 4, early stage of the AR was marked by elongation of the acrosomal tip (arrow); Fig 5, acrosome-reacting sperm showed the disrupted anterior end of the acrosome and the axial, rod-like, AP (AP); Fig 6, final stage of the AR showed the fully extended AP surrounded by the empty hull of the acrosome. Bars = 0.5 µm.

## REFERENCES


1. Touychareon S and Benchamarn P (1984) Gonadal development and sex ratio of the short-necked clam (*Paphia undulata* Born). In: *Technical paper No 35, Dept of Fisheries, Brackish-water Fisheries Division*, Thailand, pp 1-31.
2. Pongthana N (1990) Breeding and rearing of short-necked clam (*Paphia undulata*). In: *Proceeding of the Second Asian Fisheries Forum*, Tokyo, Japan, pp 635-8.
3. Dan JC and Wada SK (1955) Studies on the acrosome reaction in some bivalve spermatozoa. *Biol Bull* **109**, 40-55.
4. Niijima L and Dan JC (1965) The acrosome reaction in *Mytilus edulis* II. Stages in the reaction observed in supernumary and calcium reacted spermatozoa. *J Cell Biol* **25**, 249-59.
5. Lewis CA, Leighton DL and Vacquier VD (1980) Morphology of abalone spermatozoa before and after the acrosome reaction. *J Ultrastruct Res* **72**, 39-46.
6. Lewis CA, Talbot CF and Vacquier VD (1982) A protein from abalone sperm dissolves the egg vitelline layer by a nonenzymatic mechanism. *Dev Biol* **92**, 227-39.
7. Mozingo NM, Vacquier VD and Chandler DE (1995) Structural features of the abalone egg extracellular matrix and its role in gamete interaction during fertilization. *Mol Reprod Dev* **41**, 493-502.
8. Tilney LG, Hatano S, Ishikawa H and Mosseker MS (1973) The polymerization of actin: its role in the generation of the acrosomal process. *J Cell Biol* **59**, 109-26.
9. Tilney LG, Fukui Y and DeRosier DJ (1987) Movement of the actin filament bundle in *Mytilus* sperm: A new mechanism is proposed. *J Cell Biol* **104**, 981-93.
10. Swanson WJ and Vacquier VD (1995) Liposome fusion induced by M(R) 18000 protein localized to the acrosomal region of acrosome-reacted abalone spermatozoa. *Biochemistry* **34**, 14202-8.
11. Longo FJ (1977) An ultrastructural study of cross-fertilization (*Arbacia* x *Mytilus*). *J Cell Biol* **73**, 14-26.
12. Usui N (1987) Formation of the cylindrical structure during the acrosome reaction of abalone spermatozoa. *Gamete Res* **16**, 37-45.
13. Bozzo MG, Ribes E, Sagrista E, Poguet M and Durfort M (1993) Fine structure of the spermatozoa of *Crassotrea gigas* (Mollusca, Bivalvia). *Mol Reprod Dev* **34**, 206-11.
14. Brandriff B, Moy GW and Vacquier VD (1978) Isolation of sperm bindin from the oyster (*Crassostrea gigas*). *Gamete Res* **1**, 89-99.