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STRUCTURAL MODIFICATIONS OF SPERM FROM THE FIDDLER CRAB *UCA TANGERI* (DECAPODA) DURING EARLY STAGES OF FERTILIZATION

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ABSTRACT

The spermatozoan of *Uca tangeri* consists of a large spherical acrosome, a cup-shaped filamentous nucleus, which extends into numerous radiating arms, and a thin cytoplasmic band. Oocytes from ripe ovaries and sperm from seminal receptacles were mixed in filtered sea water, fixed at different times, and examined under a scanning electron microscope. Several spermatozoa became attached by the arms to the surface of each oocyte. The sperm bound to oocytes underwent the acrosome reaction, which started with swelling of the acrosomal vesicle followed by extrusion of its contents. Subsequently, the perforatorium projected forward ~1 μm beyond the apical end of the extruded acrosomal vesicle contents. As the ejected perforatorium becomes the leading edge of the reacted sperm, it is thought to be the constituent at which level membrane fusion between both gametes must occur.

In decapods, spermatozoa are nonmotile, aflagellate cells that display an uncondensed nucleus and one or several appendages (referred to as spikes or arms). The most distinctive feature in the spermatozoa of brachyurans is the presence of multiple arms radiating from the sperm body and a conspicuous acrosome (Brown, 1966; Pochon-Masson, 1968a; Hinsch, 1969, 1973, 1986, 1988; Reger, 1970; Jamieson, 1989a, b, 1991; Jamieson and Tudge, 1990; Felgenhauer and Abele, 1991; Medina and Rodríguez, 1992). Aside from light microscope descriptions of early researchers (Binford, 1913), detailed ultrastructural observations are available to understand the acrosome reaction and the way the sperm penetrate the egg investments (Brown, 1966; Pochon-Masson, 1968a; Hinsch, 1971; Goudeau, 1982). These previous accounts contributed substantially to understanding the processes of acrosome reaction and oocyte penetration. However, the use of the transmission electron microscope (TEM) to investigate fertilization in brachyurans meets the difficulty of the small sperm size (a few μm in diameter) in comparison to the large mature oocytes (a few hundred μm), so that the number of favorable sections is low. In contrast, scanning electron microscope (SEM) samples allow the observation of numerous sperm at different stages of fertilization.

This paper describes ultrastructural modifications undergone by the spermatozoan of *Uca tangeri* during early stages of fertilization. Some aspects of the attachment of sperm to the oocyte surface and the acrosome reaction are studied under SEM in the hope that they may serve to augment the previous reports on this subject.

MATERIALS AND METHODS

Specimens of the fiddler crab, *Uca tangeri* (Eydoux, 1835), were collected from salt marshes in the San Pedro Canal (Puerto Real, Cádiz, southern Spain) during February–June 1990. The animals were transported to the laboratory, where they were maintained in aquaria with constant running sea water. The crabs selected for electron microscope studies were used within at most 24 h after collection.

For SEM observations, solid sperm masses removed from the seminal receptacles of 3 adult females were teased apart and agitated in 10 ml of filtered sea water (FSW). After fixation with 2.5% glutaraldehyde in FSW for 2 h, the free sperm were attached to coverslips coated with 0.02% poly-L-lysine, washed in sea water, postfixed in 1% osmium tetroxide and dehydrated in acetone series. Following 2 steps of 30 min in 100% acetone, the sperm samples were critical-point dried with CO_2 in a Balzers® CPD 030 and sputter-coated with 25 nm gold in a Balzers SCD 004. Observations were made on a Jeol JSM 820 microscope. For TEM studies of unreacted sperm, small fragments of sperm masses and vasa deferentia were processed as described previously (Medina and Rodríguez, 1992).

For the study of oocyte-bound sperm, the technique developed by Clark *et al.* (1973) was applied with slight modifications. Seminal receptacles and ripe ovaries were removed from adult females during late May and June. Sperm suspensions were prepared by agitating 2 sperm masses in 10 ml of FSW. Ovaries were teased into small fragments in FSW to release free mature oocytes. The isolated oocytes were transferred to watch glasses containing only a small aliquot of FSW. Two ml of sperm suspension were added to the oocytes, at the same time providing a gyratory movement to facilitate mixing of the gametes. The process of fertilization was interrupted at various times (10 s and 30 s, and 2, 8, 10,

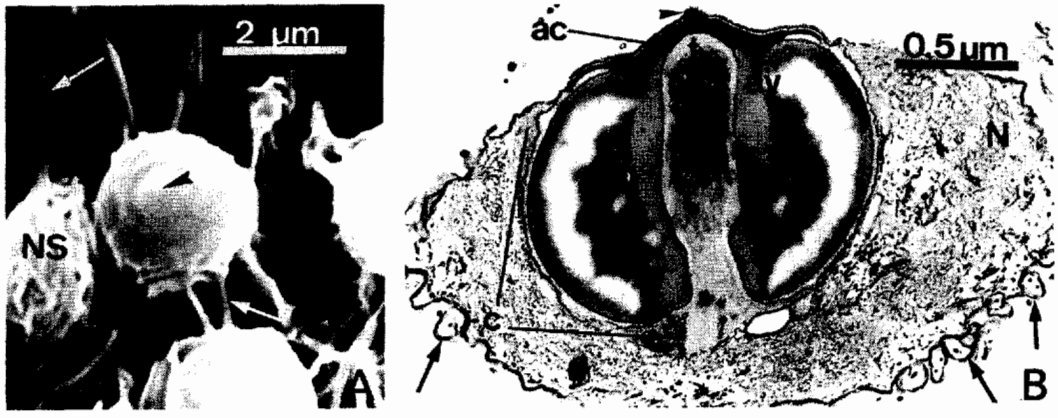

Fig. 1. Ultrastructure of mature sperm of *Uca tangeri* from the female seminal receptacle. A, SEM micrograph of sperm showing radial arms, the smooth acrosomal side with apical protuberance, and the rippled nuclear side. B, TEM longitudinal section of sperm. Arrows, sperm arms; arrowheads, apical protuberance; ac, apical cap; av, acrosomal vesicle; c, cytoplasmic band; N, nucleus; NS, nuclear side of sperm body; t, tubular formations in the perforatorium.

30, 45, 60, 90, and 150 min) by successively adding 2 ml of FSW containing 7% glutaraldehyde. Fixation in glutaraldehyde was prolonged for 3 h, after which the oocytes were washed twice in FSW, attached to coverslips and treated for SEM observation as indicated above.

Results

The spermatozoan of *Uca tangeri* is a small lens-shaped cell of 2.5–3 μm in diameter. Its upper side displays a smooth surface with a small central protuberance, whereas the lower face is markedly rippled (Fig. 1A). SEM preparations of sperm from seminal receptacles show numerous long arms (up to 5 μm in length) radiating from the sperm body (Fig. 1A). As has been shown for many other brachyurans (see Felgenhauer and Abele, 1991; Jamieson, 1991, for review), sagittal TEM sections show the spermatozoan to be mainly formed by a complex acrosomal structure partially surrounded by the cup-shaped nucleus (Fig. 1B). The acrosome consists of a large spherical vesicle that is almost completely traversed by the perforatorium (Fig. 1B). The uppermost component of the acrosomal structure is a strongly electron-dense apical cap (Fig. 1B) that shows a conspicuous central protuberance easily observed on SEM micrographs (Fig. 1A). The uncondensed nucleus displays chromatin filaments extending into the sperm arms.

After mixing of gametes in FSW, many oocytes were found to bear several sperm. The sperm attached to the oocytes with the nuclear side oriented outwards (Fig. 2A, B). The attachment area is increased by the sperm arms, some of which appear to anchor the sperm tightly to the oocyte envelope (Fig. 2B). The attachment of the spermatozoan to the oocyte surface is followed by the acrosome reaction. Ten s after mixing of gametes, nearly all the attached sperm had undergone the acrosome reaction.

After a lengthy exposure of oocytes to sperm suspensions (90–150 min), abundant reacted spermatozoa are attached to the oocyte surface, but remain entirely outside the vitelline envelope (Fig. 2C, D). This makes it possible to examine morphological changes in the sperm structure. The acrosome reaction begins with a swelling of the vesicle (Fig. 2A) followed by extrusion of its contents (Fig. 2C, D). Subsequent to the acrosomal vesicle explosion is the ejection of the perforatorium, which extends nearly 1 μm beyond the leading end of the everted vesicle contents (Fig. 2D). At this point, the nucleus often shows a central depression caused by the forward extrusion of the acrosomal structures (Fig. 2B, E). Once projected, the everted acrosomal vesicle forms a mushroom-shaped structure (Fig. 2C, D). After acrosome penetration the arms often undergo partial withdrawal (Fig. 2E). In some instances, fertilizing sperm display a filiform structure projecting from the sperm body that penetrates through the vitelline

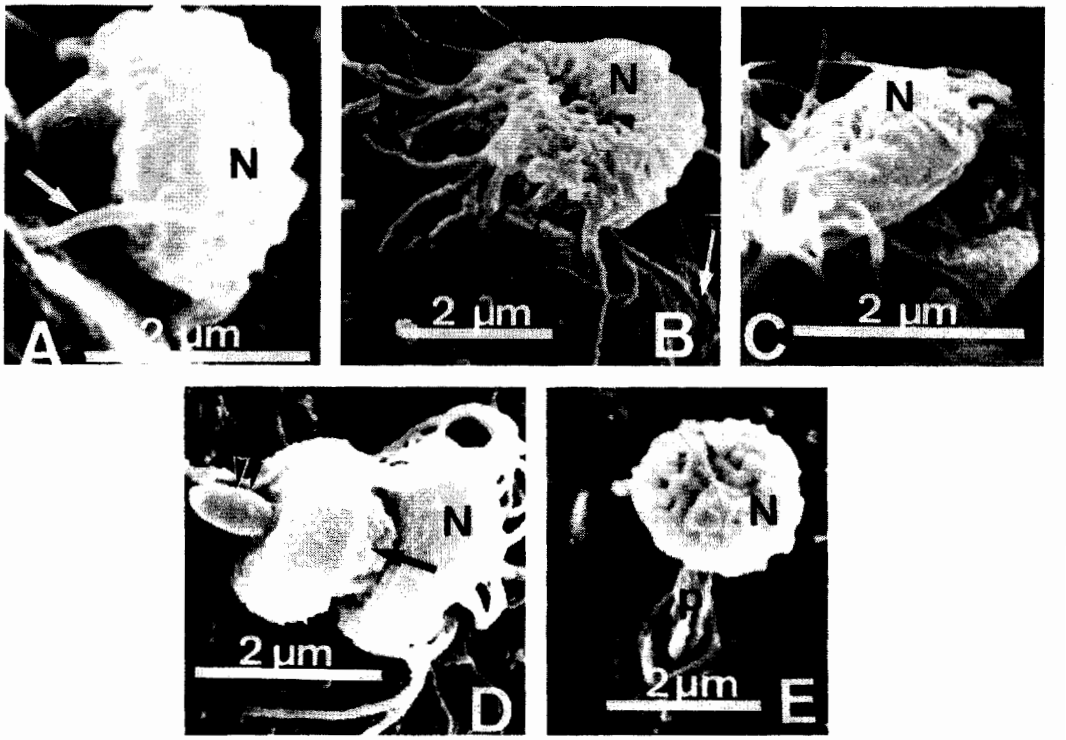

Fig. 2. SEM micrographs of oocyte-bound sperm of *Uca tangeri*. A, Initiation of the acrosome reaction (30 s). The acrosomal vesicle is swollen and the arms keep the sperm attached to the oocyte surface. B, Fertilizing sperm closely attached to the oocyte by the arms (8 min). The nucleus is slightly depressed at the center. Arrowheads indicate the site of acrosome penetration. C, Reacted sperm (150 min). The nucleus, extruded acrosomal vesicle (large arrow), and ejected perforatorium (double arrowheads) are recognized. The nuclear region has become separated from the oocyte surface as a result of the acrosome reaction. D, Reacted sperm resting on the oocyte surface (150 min). E, Fertilizing sperm (8 min) that displays the perforatorium passing through a breach in the vitelline envelope. The extruded acrosomal vesicle is no longer visible. Arrows, sperm arms; double arrowheads, ejected perforatorium; av, acrosomal vesicle; N, nuclear region; p, perforatorium.

envelope (Fig. 2E). In these sperm, the extruded acrosomal vesicle is no longer visible.

Discussion

The spermatozoan of *Uca tangeri* conforms to the general pattern of brachyuran sperm. It consists of three distinct parts: the globular acrosome, the cytoplasmic region, and the uncondensed cup-shaped nucleus with radiating arms. Sperm arms are present in all brachyuran species studied with the exception of *Petalomera lateralis* (see Jamieson, 1990). They may be packed with microtubules (Pochon-Masson, 1965a, 1968a; Hinsch, 1969, 1973) or lack them (Hinsch, 1986, 1988; Jamieson, 1989a, b; Jamieson and Tudge, 1990). The isolated sperm of *U. tangeri* possess numerous radial arms devoid of microtubules (Medina and Rodríguez, 1992). These appendages bring the sperm into contact with the oocyte (Brown, 1966; Hinsch, 1971). This seems likewise to be one of the roles of the sperm arms in *U. tangeri,* since they bind to the vitelline envelope along their whole extension. They do not appear to perform any specific function other than that of sperm binding; after the acrosome reaction they often undergo withdrawal and sometimes are no longer recognizable.

After sperm attachment to the oocyte surface, the acrosomal vesicle undergoes swelling, followed by extrusion of its contents. Acrosomal vesicle extrusion appears to be rapid, since incomplete acrosome reactions like that shown in Fig. 2A are rarely found even in the earliest fertilized oocytes. The extrusion of the acrosomal vesicle is considered to be a passive mechanism resulting

from hydration of the inner substance in response to varying artificial inducers, e.g., decreased tonicity of the medium (Pochon-Masson, 1965b, 1968a, b) or action of ionophores (Talbot and Chanmanon, 1980). In natural conditions, the flow of water into the acrosomal vesicle is most probably due to a change in membrane permeability as a result of the interaction between the sperm plasma membrane and the egg vitelline envelope.

A recent work on in vitro fertilization in the lobster *Homarus americanus* has shown that the extruded acrosomal vesicle materials remain for a while in the perivitelline space (Talbot *et al.*, 1991). It is plausible that this is also true for *U. tangeri*, since the extruded acrosomal vesicle contents of sperm located outside the vitelline envelope form a persistent, mushroom-shaped structure. Hinsch (1971) suggested that sperm penetration is facilitated by a lytic action of the acrosomal contents on the vitelline envelope. However, no evidence of digestive activity has been found in *H. americanus* (Talbot *et al.*, 1991).

Despite the excellent electron microscopical works available on decapod fertilization (Brown, 1966; Hinsch, 1971; Goudeau, 1982; Lynn and Clark, 1983; Talbot *et al.*, 1991), a crucial event such as membrane fusion of both gametes has never been seen. Nevertheless, the close contact of the perforatorium with the oocyte membrane during fertilization (Brown, 1966; Goudeau, 1982), as well as the observation that in *H. americanus* the acrosomal filament is not surrounded by membrane after penetration into the ooplasm (Talbot *et al.*, 1991), suggest that membrane fusion could happen at this level. The inner ultrastructure of the perforatorium remains nearly unchanged after acrosome reaction in the crabs *Callinectes sapidus* (see Brown, 1966) and *Carcinus maenas* and *Cancer pagurus* (see Pochon-Masson, 1968a), although elongation appears to occur (Pochon-Masson, 1968a). This is consistent with the present electron microscope observations in *U. tangeri*, where the perforatorium is projected forward and becomes the leading end of the reacted sperm. TEM studies are currently underway in an attempt to determine the causes of the ejection of the perforatorium during the acrosome reaction.

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