THE MANNER OF SPERM ENTRY IN VARIOUS MARINE OVA

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(With Eleven Text-figures.)

This paper is a record of observations on insemination in five species of marine forms, Arbacia punctulata (sea urchin), Woods Hole, Mass., Paracentrotus (Strongylocentrotus) lividus (sea urchin), Villefranche-sur-Mer, Echinarchnium parma (sand-dollar), Mt Desert Island and Woods Hole, Cerebratulus lacteus (nemertine), Mt Desert Island and Woods Hole, and Nereis limbata (annelid), Woods Hole. The observations on all except the European species were made at different times during several summers.

I. THE JELLY AROUND THE EGGS OF ARBACIA AND ECHINARCHNIIUS.

The clear jelly which surrounds the unfertilised eggs of Arbacia and Echinarchniius offers no obstacle to the narrow, tapering heads of the sperm of either species. It does serve as such for the blunt-headed sperm of Asterias(1). In Arbacia the "jelly" is a fibrillar network with loose meshes except at the periphery where the fibrillae are closely matted together. This can be detected by immersing the eggs in a suspension of India ink or in a heavy suspension of spermatozoa. The ink particles and the spermatozoa collect about the egg in two concentric regions, one on the surface of the egg and the other at the periphery of the network where they are entangled by the matted fibrillae.

In Echinarchniius the jelly is relatively dense and more uniform in texture and is similar to that of the Asterias egg except for the distribution throughout its substance of minute, reddish pigment cells. The jelly when shaken or torn breaks off in lumps which persist for a long time in sea water, a property quite different from the readily disintegrating meshwork of the "jelly" of the Arbacia egg.

II. INSEMINATION IN ECHINARCHNIIUS, ARBACIA AND PARACENTROTUS.

These three species represent the type in which no localised elevation of the egg surface occurs at the site of entry until after the sperm head has passed completely into the egg, hence the term, "cone of exudation," first described by Selenka(2), and Fol(3), for a closely allied genus, Toxopneustes, and by Wilson and Mathews for Arbacia(4).
The several steps in the insemination of *Echinorachnius* are shown in Fig. 1 (a–m). Not every spermatozoan which touches the egg necessarily penetrates. Frequently, the spermatozoa glide along the surface of the egg and move away, some having remained there for several seconds, poised with the tips of their heads touching the egg. In the case of those which penetrate, the tip of the sperm head first adheres to the surface of the egg and, in many cases in which the tail is actively vibrating, the head rotates several times about its tip before entering (Fig. 1, a–d). The adhesion to the egg can be detected by touching the sperm with a micro-needle. If there is no adhesion the sperm moves quickly away, otherwise it cannot be dislodged.

![Fig. 1. Various stages in the insemination of *Echinorachnius parma*. a–d. Sketches illustrating the revolution of sperm head about its tip as pivot immediately after the tip becomes attached to egg. e–g. Sperm head penetrating the floor of a slight depression in egg. Note recession of cytoplasmic granules and lifting of fertilisation membrane. h. Rising of exudation cone after completed sperm entry. i–k. Breaking in two of tail from pull caused by continued lifting of membrane. Note conversion of exudation cone into irregular flame-like processes. l. Recession of flame-like processes and beginning of the spread of hyaline membrane over egg from their base. m. Six minutes later, showing appearance of sperm aster.](image-url)

The response of the egg to the entering sperm is as follows: The surface of the egg, to which the sperm adheres, forms a shallow depression where a membrane (e) lifts off, and slips over the head (f). Delicate strands of cytoplasm usually stretch between the rising membrane and the egg's surface; these strands finally break into droplets and disappear. Just below the depression the cytoplasm becomes hyaline with a disappearance of its granules (e, f, g). The fertilisation membrane begins to rise a few seconds after the spermatozoan has touched the egg and spreads from this spot over the surface of the egg in about 40–50 sec. as Just (5) has already described.

In the *Echinorachnius* egg the actual penetration of the sperm head is sometimes delayed for as long as 1 min. during which time it continues its spasmodic oscillations about its tip. As soon as penetration begins, the side-to-side motions of the
head stop, although the tail continues to lash to and fro. The sperm head then sinks quickly into the egg along an ever deepening hyaline path, with a peculiar gliding motion. Within 6–7 min. the diminutive sperm aster (m) becomes visible at the inner end of the hyaline path.

After complete penetration the hyaline exudation cone forms with continuously changing flame-like processes (k–k), which repeatedly grow out and withdraw for 8–10 min. after insemination. During this time the hyaline base of the cone becomes sharply differentiated from the granular cytoplasm and spreads as a delicate hyaline layer (k–m) over the egg. This is probably the initiation of the so-called hyaline plasma layer or membrane (6, 7).

By means of the micro-dissection needle it can be determined that the fertilisation membrane stiffens as it rises off the surface of the egg, a fact already noted by Hobson (8). During the stiffening process the tail of the sperm tends to be caught and broken by the lifting membrane. The proximal part of the tail then becomes lost among the processes of the exudation cone (Fig. 1, h, i, j), while the distal end persists for many minutes, lying motionless outside the membrane (k, l, m).

The extent to which the tail passes through the fertilisation membrane depends upon the rapidity with which the membrane rises. A slowly rising membrane grips the tail before it has gone very far. If the rise is rapid, more of the tail slips through before being caught. An interesting case is shown in Fig. 2 in which the tail was gripped close to the head (a). As the membrane rose the proximal part of the tail stretched (b, c), while the portion outside the membrane continued lashing about. Finally (d) the tail broke, whereupon the part outside the membrane stopped moving while the portion still in contact with the head was now free and exhibited a lashing movement. Thirty seconds later (e, f) this portion also stopped moving and disappeared among the processes of the exudation cone.

Fig. 3 records a case in which the spermatozoon was unusually active. The fertilisation membrane rose so rapidly that the entire length of the sperm tail came to lie within the membrane. The sperm head, instead of assuming the usual and apparently passive glide while passing into the egg, kept up an active burrowing motion and carried its tail with it (b, c, d). Within the egg the tail continued
its lashing movements and 3 min. later the spermatozoon had left the hyaline pathway (e), and wandered among the cytoplasmic granules where its movements were visible by the jostling about of the granules. One minute later (i.e. 5 min. after insemination) it stopped moving and became lost to view. At 6½ min. a diminutive sperm aster (f) appeared where the sperm head had last been seen. At 8 min. the aster had enlarged considerably and started to migrate toward the centre of the egg. The exudation cone progressively diminishes but is often detectable for 8–10 min. after insemination.

The following case is of interest to show that the fertilisation membrane may remove a superfluous spermatozoon even after there are unmistakable signs of imminent penetration. The egg had been fertilised and the fertilisation membrane was visible over about half the egg (Fig. 4, a). A second spermatozoon adhered to the surface of the egg beyond the lifted membrane, resulting in an indentation of the egg’s surface and the initiation of a hyaline path in the cytoplasm (b). One minute later, the hyaline region had enlarged and the head of the spermatozoon was actively oscillating about its tip, as if preparing to enter (e). However, during the following minute, the slowly lifting membrane spread over this spot (d), and lifted off the spermatozoon (e). In d and e the cytoplasmic granules are shown returning and obliterating the hyaline region which had been formed anticipatory to sperm entry.

In Arbacia the process of insemination is similar but more rapid than in Echinarchaeus.

Immature eggs (with germinal vesicles) of Arbacia and of Echinarchaeus readily receive their respective spermatozoa. No fertilisation membrane is lifted off but for each entering spermatozoon a prominent exudation cone is formed with unusually large and long, flame-like processes. Both in mature and immature eggs the surface
of these processes is extraordinarily tough when manipulated with micro-needles. This property provides additive evidence that the hyaline plasma membrane, of a similar toughness, is initiated with the formation of the exudation cone.

The insemination of *Paracentrotus* was observed at Villefranche-sur-Mer on the Mediterranean. During the penetration of the spermatozoon the part of the head still outside the egg underwent changes in shape somewhat like those described for the starfish sperm (1).

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**Fig. 4.**

- *a.* Echinarchaeus egg fertilised and showing a slowly lifting membrane. A second spermatozoon about to enter.
- *b, c.* Details of effect on egg by the second spermatozoon.
- *d, e.* Second spermatozoon being lifted off by advancing membrane.

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**III. INSEMINATION IN CEREBRATULUS.**

The insemination of the *Cerebratulus* egg is difficult to observe because of the lack of obvious reactions which, in other eggs, help one to identify the spermatozoon responsible for fertilisation.

No fertilisation membrane is formed nor is there any nipple-like elevation either during or after sperm entry. This was remarked upon by Yatsu (6). The egg is too opaque to permit intra-cytoplasmic observations except when considerably compressed and there is no immediate visible change in the contour of the egg at the site of sperm entry. However, several seconds after penetration, convulsive movements of the egg start in the vicinity where the sperm had entered and gradually extend in scope so as to produce a considerable distortion of the egg. Some time before cleavage the egg returns to its spherical shape.

The eggs are passed into the sea water during the germinal vesicle stage. Contact with sea water initiates the elevation of a vitelline membrane, the space between it and the surface of the egg progressively widening until within an hour or so it is broader than half the diameter of the egg (9). The egg at first is somewhat broad pear-shaped but soon rounds off except for a narrow conical stalk, the place of former attachment in the ovary. The vitelline membrane extends over the sides of the stalk but is deficient at the tip.
By the time the polar bodies begin to form, the cytoplasmic stalk is almost entirely withdrawn leaving a conical projection of the vitelline membrane open at the tip and simulating a micropyle. However, the spermatozoa can pass through the vitelline membrane with ease and insemination may take place anywhere. The germinal vesicle is normally eccentric and so situated that the polar bodies are formed opposite the "micropyle." Coe (10) has commented on the frequency of insemination at the pole opposite the polar bodies where the deficiency of jelly and membrane at the region of the micropyle offers an unimpeded route for those spermatozoa which chance to use it.

The spermatozoa have long, narrow, sickle-shaped heads. When in motion the path taken is a series of sweeping curves, but, by an occasional jerk of the head, the direction of the path tends to alternate in opposite directions. The peculiar whip-lash movement of the tail is best understood from the figures. In Fig. 5 is
shown successive stages of sperm entry in an egg which previously had been
denuded of its vitelline membrane. The tail is extremely active during penetration,
and the impression is gained that the sperm is boring its way in. After the head
has entered the movement of the tail may slacken for a time and then frequently
show renewed activity.

The curved path taken by the sperm frequently brings about untoward results.
After passing through the vitelline membrane the sweeping curves often cause the
sperm to miss the egg’s surface and to carry them round and back to the inside of
the membrane with their tips directed away from the egg (Fig. 6). This happens
especially with eggs which have been lying in sea water for an hour or more so
that the space between the vitelline membrane and the egg’s surface is considerable.
Fig. 7 shows a successful insemination in which the sperm had struck the vitelline
membrane at the proper angle so that further progress brought the tip on the
surface of the egg.

Fig. 8 shows a case in which the head, by revolving on its axis, alternately
stretched and slackened that part of its tail held within the membrane.

Not every spermatozoon penetrates after touching the egg’s surface. In many
cases a sperm will remain poised for many seconds with its tail apparently exerting
its full driving force and then will suddenly or gradually move away, sometimes
disappearing to be found again lying lengthwise below the horizon of the egg and
out of focus.

A sperm never penetrates unless its tip adheres to the surface of the egg, and
its progress, when once started, is a peculiar glide, as if the head were being sucked
in by the egg.

IV. CEREBRATULUS SPERM AND ECHINARACHNIUS EGGS.

*Cerebratulus* sperm penetrate the cortex of *Echinarachnius* eggs with ease. There
is no typical fertilisation response and the eggs can be self-fertilised either at the

![Sperm penetration](https://via.placeholder.com/150)

...same time or subsequently, and will undergo normal development with their
cortical zones riddled with *Cerebratulus* sperm. Penetration appears to be due to
a boring action of the sperm. When once the sperm are inside the egg, vacuolar
spaces form and fuse together so as to enclose the sperm, many of which remain
actively moving for hours (Fig. 9).
The Manner of Sperm Entry in Various Marine Ova

During the penetrating process an extraordinary phenomenon can sometimes be observed. This is shown in Fig. 10. During the first minute the violent lashing of the tail forced the greater part of the sperm head into the egg. During the next 2 min. the tail became lax and motionless. The head was then extruded as if it were being squeezed out by the egg. Thereupon the tail again lashed about and the head began to move in. After a time the tail again relaxed and the head was for a second time extruded. This procedure was repeated once more, but finally the sperm was completely extruded and further attempt at penetration ceased.

![Fig. 10. Successive steps in repeated attempts of Cerabronulus spermatozoon to push its long conical head into an Echinus echinus egg. With each relaxation of the tail the head became partially and finally completely extruded.](image)

V. INSEMINATION IN NEREIS.

As is known from the classical work of F. R. Lillie, the unfertilised Nereis egg possesses a vitelline membrane sub-adjacent to which is a cortical, coarse alveolar layer. After fertilisation the contents of the alveoli are extruded through the vitelline membrane to form a considerable zone of jelly surrounding the egg. The formation of the jelly is complete except at one spot on the membrane occupied by the fertilising spermatozoon. The perivitelline space, which now appears underneath the vitelline membrane, is, according to Lillie, *intra-ocular*, i.e. *intra-cytoplasmic*, with contracted alveoli filled with fluid and with a delicate cytoplasmic wall lining the vitelline membrane.

In Fig. 11, a, b, is shown a micro-needle with its tip passing through diffraction rings and impinging on the true surface of the egg. The cortex, traversed by numerous radiating strands, is stiff and, when indented with a needle, bears down on the softer granular cytoplasm underneath.

After fertilisation the exudation of the jelly through the investing membrane converts the cortex into a space traversed by radiating protoplasmic strands which are fewer than those seen in the unfertilised egg. The membrane now can be indented without distorting the main body of the egg beneath (Fig. 11, c, d).

An interpretation alternative to that of Lillie for the structure of the cortex of the unfertilised egg is that the jelly, instead of being intra-alveolar, is extra-protoplasmic and lies between numerous protoplasmic processes or strands which extend from the surface of the egg to the enveloping vitelline membrane. When the jelly is extruded upon fertilisation the radiating protoplasmic strands are eventually withdrawn so that the surface of the egg becomes evenly contoured with a space between it and the vitelline membrane.
The extrusion of the jelly occurs while the spermatozoon, responsible for the fertilisation, is attached to the vitelline membrane. According to Lillie, a transparent cone rises from the protoplasmic surface of the egg, crosses the perivitelline space, and becomes attached to the vitelline membrane beneath the spermatozoon. This recalls Fol's "cone d'attraction" in *Asterias* ova. A re-examination of this phenomenon makes it highly probable that the spermatozoon, when making contact with the surface of the egg, becomes attached at the start to one or several of the numerous cytoplasmic strands which extend to the investing membrane through the jelly-filled space. When the jelly is extruded the strands connected with the sperm persist and their bases broaden to form shallow conical elevations. The broadening process extends along the strands until a prominent cone or column reaches the head of the spermatozoon.

This phenomenon recalls that of the starfish egg, except that in the latter there is no membrane investing the jelly and the strand to which the sperm becomes attached does not enlarge before shortening to draw the sperm to the egg. In *Nereis*, on the other hand, the sperm which has a broad ovoid head, remains on the external surface of the vitelline membrane until a flow of cytoplasm enlarges the strand. Analogous extended enlargements of the entrance cone and its filamentous extension occur in the starfish egg in cases where the spermatozoon is prevented from being readily pulled to the egg.
As already shown by Goodrich (13), the spermatozoon, during the early stages of insemination, can be removed readily with a micro-needle. Later, while still on the surface of the vitelline membrane, it is firmly attached. With a needle its head can be pulled out into a long, slender ribbon which, on being released, returns to the original shape and position. It can be removed by breaking its attachment, whereupon the cone gradually sinks back into the egg.

The passage of the sperm head through the vitelline membrane into the enlarged entrance cone can be readily seen in the living state, as described by Lillie (13), in fixed material. The head narrows considerably in passing through and becomes long drawn out within the cone. The narrowing of the head when passing through the relatively stiff membrane recalls the similar phenomenon in the starfish egg, the sperm of which reaches the fertilisation membrane sometime after it has begun to rise off the egg. The middle piece is discarded in a manner similar to that occasionally observed in the starfish.

An additional similarity to the starfish egg is the fact that when the lengthened cone with its contained sperm begins to be withdrawn into the egg there is a similar withdrawal of all the cytoplasmic extensions in the perivitelline space so that the surface of the fertilised egg finally becomes evenly contoured.

VI. DISCUSSION.

In a study of insemination of the various species one is struck with the lack of any directional movement of the spermatozoa. This was pointed out long ago by Buller (14). The only visible response of the spermatozoa to the presence of the eggs seems to be an increased activity in their random movements. The stickiness of the jelly surrounding the eggs tends to trap them and, in the species described in this paper, many pass through the jelly to the surface of the eggs. There they may as likely turn away as adhere and accomplish fertilisation. A visible response of the egg seems to occur only after a spermatozoon has made actual contact with some part of the cytoplasmic surface of the egg. Admission is not haphazard, for many spermatozoa may encounter an egg before penetration occurs. Also a single spermatozoon may wander from one Arbacia egg to the other and only inseminate the last egg encountered.

There are several points of interest concerning the nature of the tail of the spermatozoon. It can be stretched considerably without breaking, cf. Fig. a, b, c. It is elastic as shown by the fact that the tail can be made repeatedly to stretch and return to its original length, cf. Fig. 8. Finally, when a tail is broken in two the portion attached to the head of the sperm maintains its normal activity while the other portion instantly stops moving, cf. Fig. 2, d.

In regard to the movement of the tail (Fig. 2, b, c) there seems to be no need for a propagation of a wave of motion from the base of the tail in order that the tip may move. In the case illustrated the lifting fertilisation membrane, as it stiffened, gripped the tail by the middle and stretched it so that the proximal portion was held motionless. In spite of this the distal end kept up its lashing movements.
The adhesion of the head of the spermatozoon preparatory to penetration need not signify fusion of the protoplasm of egg and sperm. The fact that a spermatozoon with its tail can enter an egg (cf. Lillie(15)) and maintain sufficient organised individuality to keep on wriggling about within the cytoplasm of the egg (Fig. 3, b, c), argues against the idea that the plasma membranes of egg and sperm fuse when they first come into contact. The dissolution of the plasma membrane of the sperm to free its nucleus probably takes place after the spermatozoon is well within the egg.

The process of fertilisation frequently involves changes in surface contours. For example, the body of the spermatozoon in the starfish(1) and in Paracentrotus undergoes repeated distortions in shape during penetration. In the case of the egg may be cited the retraction of filamentous processes in the starfish (1) and in Nereis. More extensive reactions of the egg's surface are illustrated by the convulsive movements of the nemertine egg which take place for some time after sperm entry. This resembles the distortion of the Cestumna egg described by Morgan and Tyler (16).

In Echinoderm eggs the recession of the flame-like outgrowths leaves a fine, stiff membrane, the development of which starts at the site of sperm entry and spreads over the surface of the egg. This membrane is better developed in some species than in others and has long been known as the hyaline plasma layer or membrane. It serves to hold the blastomeres together (7). The flame-like processes are extraordinarily large in the immature Arbacia egg and were described by Seifriz(17), who noted their appearance when sperm and eggs are mixed but did not realise that they result from sperm penetration and as such are analogous to those occurring in mature eggs.

The observations on the insemination of the Nereis egg suggest a similarity to that of Asterias. In both eggs a nipple-like elevation forms on the surface of the egg, apparently at some distance from the spermatozoon. In both cases the nipple appears to be the broadened base of a filament the other end of which is attached to the spermatozoon before the nipple appears. In the starfish the filament shortens and draws the attached spermatozoon with it into the egg(1). In Nereis, where there is a broad perivitelline space, the spermatozoon remains outside the vitelline membrane and a flow of cytoplasm from the nipple enlarges the strand and converts it into a column large enough to engulf the head of the spermatozoon. A somewhat similar phenomenon was described long ago by Calberla(18) for Petromyzon.

The reaction recorded in this paper of the sanddollar egg to Cerebratulus sperm furnishes an additional mechanism whereby cross-fertilisation is prevented. In this case the cytoplasm of the egg reacts to the presence of the foreign sperm by walling them off within vacuoles. This reaction does not prevent the egg from becoming fertilised subsequently with its own sperm.
REFERENCES.