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BEHAVIOR OF SPERM NUCLEI IN MEIOTIC EGGS OF THE OYSTER, *CRASSOSTREA GIGAS*[1]

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The behavior of sperm nuclei incorporated in oyster oocytes was examined by fluorescence microscopy. The spermatozoa seemed to enter the first metaphase oocytes at any site along the egg surface independent of the animal-vegetal polarity. The incorporated sperm nucleus remained in the egg cortex until the completion of egg meiosis. The female pronucleus migrated toward the male pronucleus locating in the periphery of the zygote. The associated pronuclei moved to the center of the zygote, where the mitotic aster began to develop.

Oyster oocytes dissected from ovaries are at the meiotic prophase (the geminal vesicle stage). The oocytes having the germinal vesicle (the GV oocytes) undergo germinal vesicle breakdown (GVBD) by the application of serotonin and changed to the first mataphase of meiosis, at which they were arrested. These first metaphase oocytes (the metaphase I oocytes) are fertilized by artificial insemination and proceed to embryogenesis (OSANAI, 1985). The chorion enveloping GV oocytes is removed by being exposed to trypsin in acid Ca, Mg-free sea water without spontaneous GVBD and oocyte activation (OSANAI and KYOZUKA, 1988). The improvement of the procedures allowed us immunofluorescence microscopic study in oyster oocytes (OSANAI and KURAISHI, 1988). Using the improved methods, we observed the meiotic changes and the behavior of sperm nuclei in oyster zygotes.

MATERIALS AND METHODS

Gamete

The oyster, *Crassostrea gigas*, were collected near the Marine Biological Station, Asamushi, Aomori and kept in running sea water. GV oocytes were obtained by dissecting ovaries in acid sea water (pH. 6). To remove the chorion the oocytes were incubated in acid Ca, Mg-free sea water (pH 6.0) containing 1 mM ethyleneglycol-bis-(β-aminoethylether) N, N′-tetra-acetic acid (EGTA) and 0.2 mg/ml trypsin for 10 min. The dechorionated oocytes were reincubated in sea water containing 1 or 10 μM serotonin (5-hydroxytryptamine) creatine sulphate for 30-50 min. The

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oocytes underwent GVBD and were arrested at the first metaphase of meiosis. The metaphase I oocytes were inseminated in ordinary sea water and used for cytology and fluoresence microscopy.

Fluorescence microscopy

Dechorionated oocytes were incubated 1 μM serotonin in sea water for 30 min. The metaphase I oocytes obtained were inseminated in sea water and allowed to develop at 20°C. At 5–60 min after insemination the zygotes were incubated in the extraction medium (Osanai and Kyozuka, 1988) for 1–2 hrs and then fixed in cold methanol for 15 min. The fixed specimens were rinsed with borate-buffered saline (BBS, pH 8.1) and adhered to a polylysin-coated glass coverslip. After rinsing with BBS containing 0.05% Triton X-100 (Triton-BBS) the coverslip was incubated in BBS containing monochronal anti-tubulin antibody (mouse) (Amersham) for 1 hr. The specimen was rinsed with Triton-BBS and stained with fluorescein isothiocyanate-labelled anti-mouse IgG (goat) (Tago Co.) for 60 min. After rinsing with Triton-BBS, the specimens were stained in BBS containing 0.05 mg/ml 4′, 6-diamino-2-phenylindol dihydrochloride (DAPI) for 20 min and then mounted on a glass slide with glycerin.

Staining with lacto-aceto-orcein

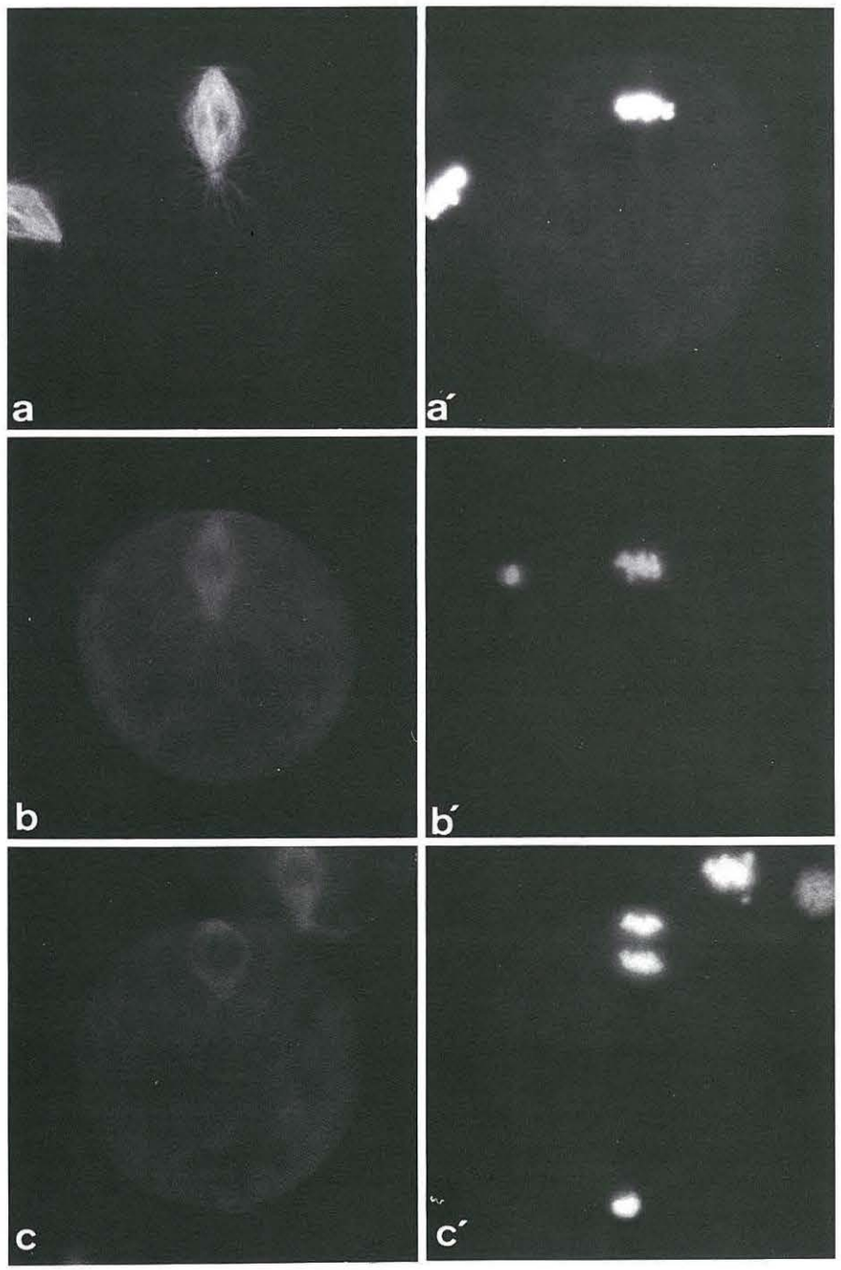
Dechorionated oocytes were incubated in 10 μM serotonin for 50 min. The metaphase I oocytes were inseminated and fixed in Carnoy's methanol-acetic acid (3 : 1) overnight after the incubation for 0–45 min at 25°C following insemination. Fixed specimens suspended in Carnoy's fluid were dropped on a glass slide and covered with lacto-aceto-orcein solution. After 10 min the specimens were somewhat compressed with a cover slip and were sealed with nail polish.

Results

In the unfertilized oocytes (the metaphase I oocytes) the first meiotic spindle located near the animal pole. The spindle axis was perpendicular to the contacting surface of oocyte (Fig. 1a). The chromosomes arranged on the metaphase plate (Fig. 2a).

The zygotes were yet at the first metaphase of meiosis 5 min after insemination at 20°C. Fertilization was monospermic also in the dechorionated oocytes. The incorporated sperm nuclei located in the whole cortex. Spermatozoa seemed to

Fig. 1. Immunofluorescence microscopic photographs of oyster zygotes. Dechorionated metaphase I oocytes were inseminated and allowed to develop in sea water at 20°C. a, a′ : First metaphase of meisis before insemination. b, b′ : First metaphase of meiosis, 5 min after insemination. c, c′ : First anaphase of meiosis, 10 min after insemination.



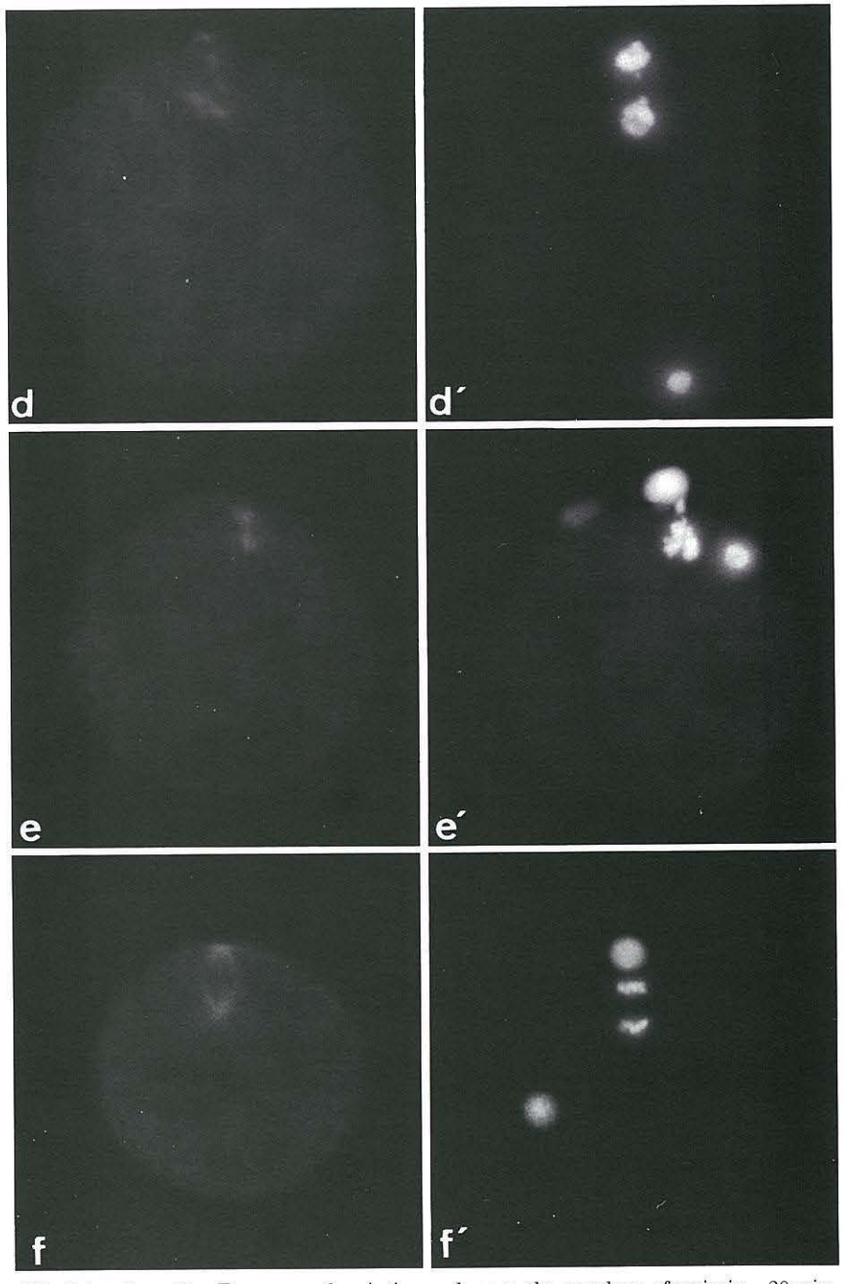


Fig. 1 (continued). From second meiotic prophase to the anaphase of meiosis. 20 min (d, d′) and 30 min (e, e′, f, f′) after insemination.

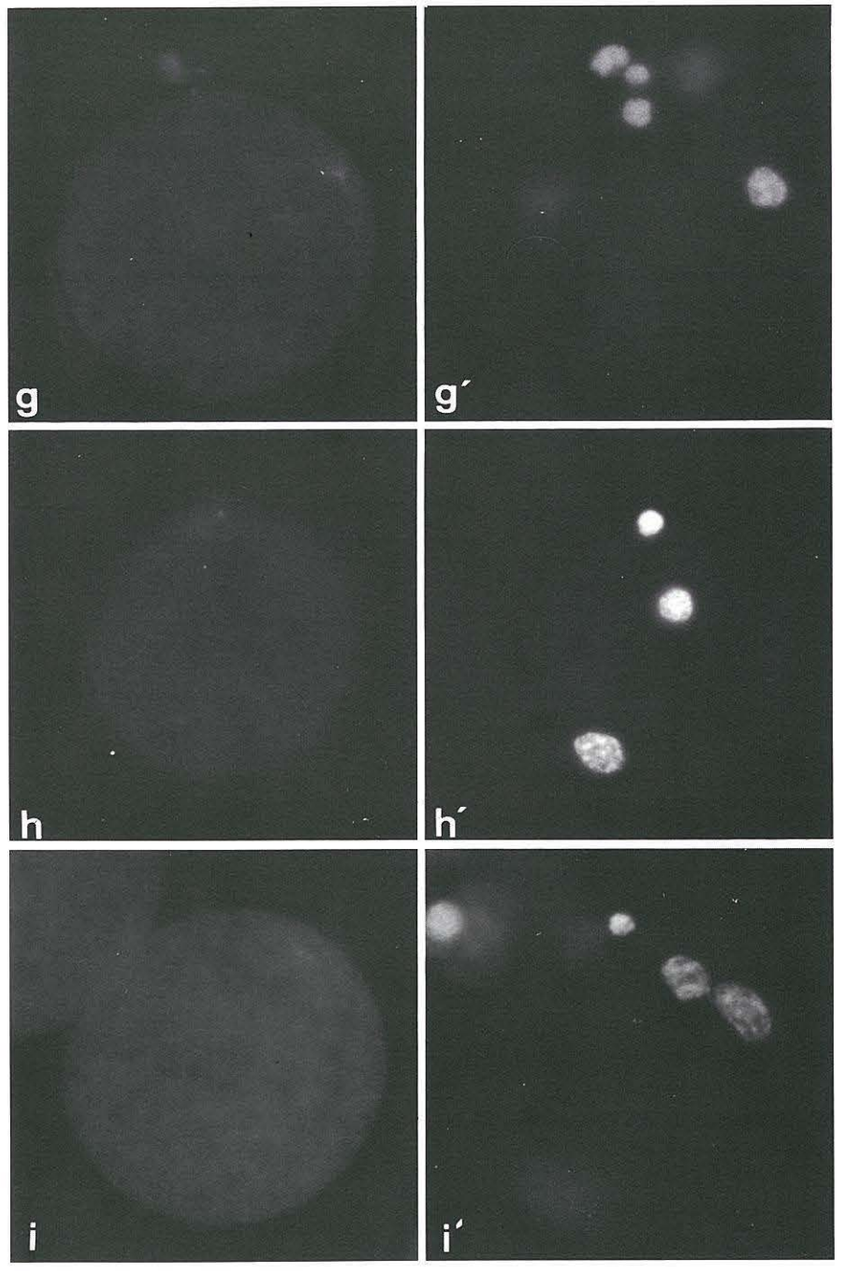


Fig. 1 (continued). From the completion of meiosis to the association of pronuclei, 40 min (g, g′, h, h′) and 50 min (i, i′) after insemination.

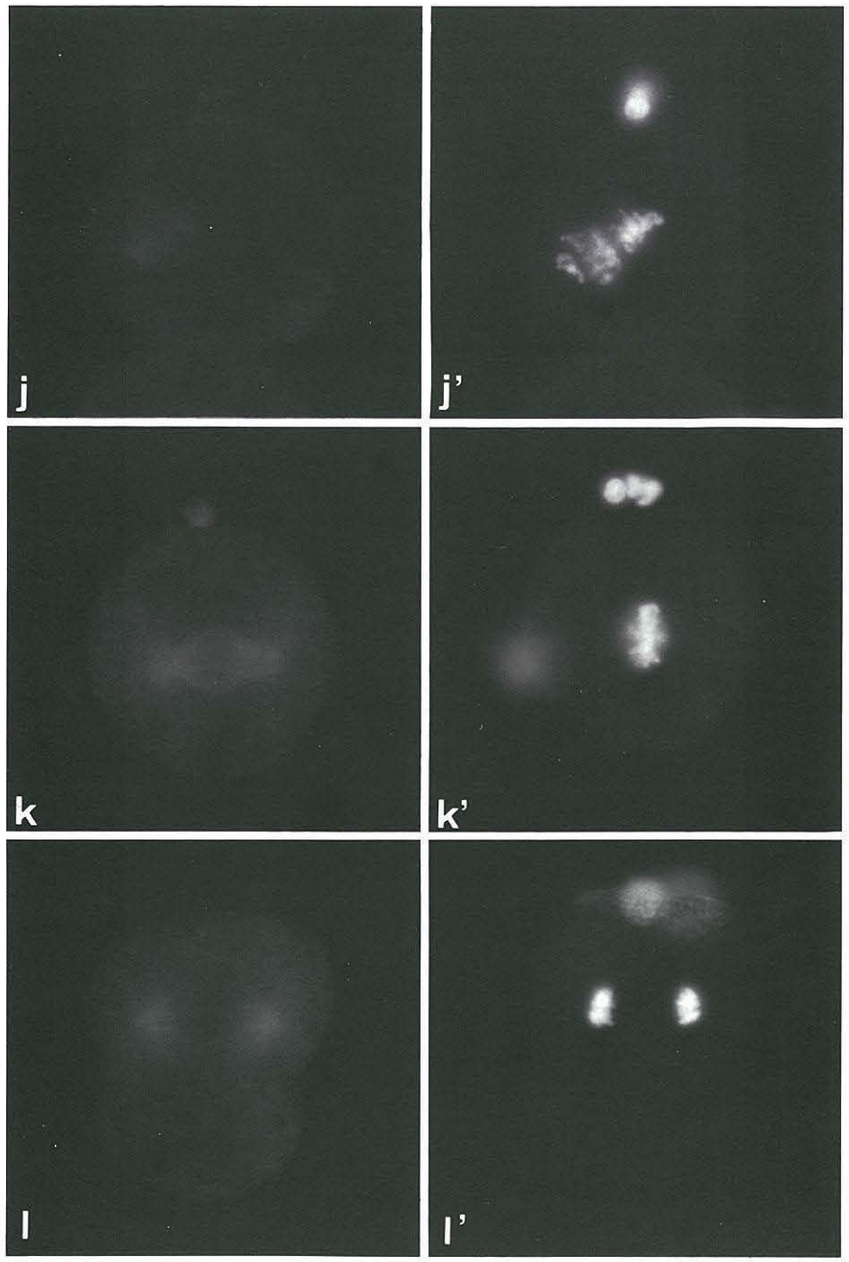


Fig. 1 (continued). From the separation of mitotic centers to the anaphase of first mitosis. i-l, j-l′, 70 min after insemination. a-l, stained with anti-tubulin antibody. a′-1′, stained with DAPI. ×810.

enter at any site along the oocyte surface independent of the animal-vegetal polarity. The incorported sperm nuclei somewhat swelled, but retained a condensed state in the periphery of the zygotes (Fig. 1b, b′). No bright spot corresponding to the sperm aster was observed around the sperm nuclei.

Meiotic division resumed 10 min after insemination. Dyads separated toward the spindle poles (the first anaphase of meiosis). The condensed sperm nucleus located yet in the cell periphery (Fig. 1c, c′).

At 20 min after insemination, the first polar body was extruded. The trace of the first meiotic spindle remained near the animal pole. A new spindle having a

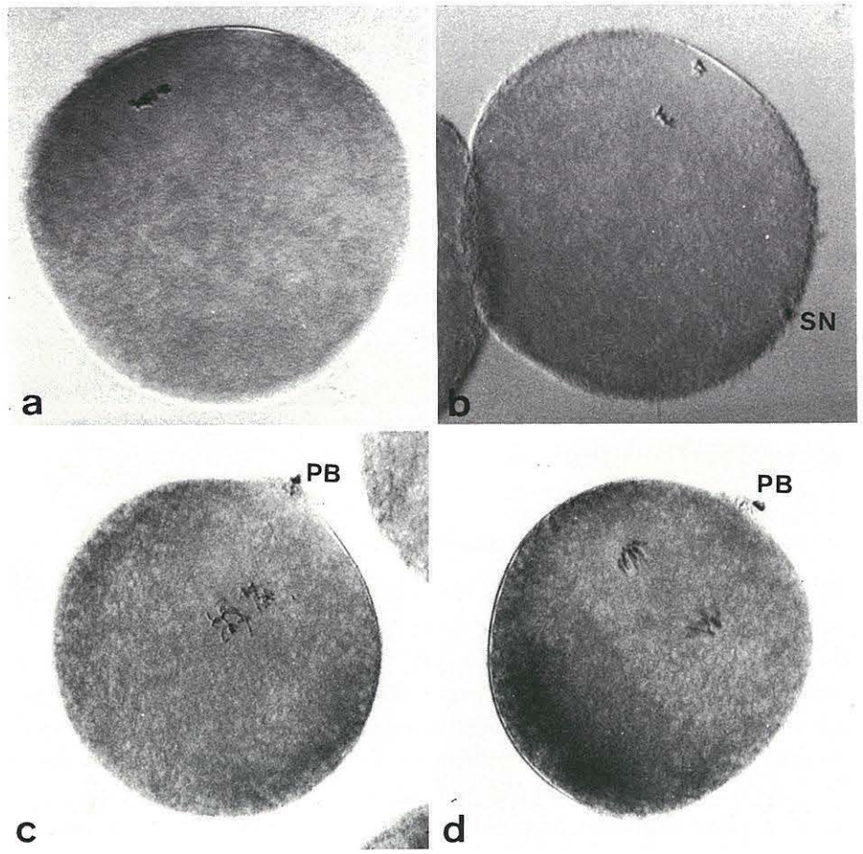


Fig. 2. Nuclear changes from metaphase I to first mitosis. Dechorionated metaphase I oocytes, were inseminated and placed in sea water at 25°C. a : Metaphase I oocyte before insemination. b : Second metaphase of meiosis, 15 min after insemination. c : Pronuclei, 30 min after insemination. d : First mitosis, 45 min after insemination. PB, polar body ; SN, sperm nucleus. Stained with lacto-aceto-orcein. Photographed with a Nomarski differential interfernce microscope. ×550.

pair of bright poles appeared near the centripetal side of the dyad nucleus remained in the zygote. The spindle axis was oblique to the animal-vegital axis. The sperm nucleus located in the cortical region (Fig. 1d, d′).

At 30 min after insemination, the zygotes were at the prophase or metaphase of meiosis. The spindle axis became to be parallel to the animal-vegetal axis (Fig. 1e, e′). The chromosomes aligned in the metaphase plate (Fig. 2b), and the monads separated toward the spindle poles (Fig. 1f, f′). The sperm nucleus retained the condensed state in the cortical region of the zygote (Fig. 1e, e′, f, f′).

After incubation for 40 min at 20°C, the second polar body was extruded from the animal pole. The chromosomes remained in the zygote developed into the female pronucleus. The sperm nucleus began to swell in the egg periphery. A samll aster consisted of one bright spot appeared near the swelling sperm nucleus (Fig. 1g, g′). When the female pronucleus began to migrate toward the male pronucleus, the growing aster was observed near the male pronucleus, but not near the migrating female pronucleus (Fig. 1h, h′).

At 50 min after insemination, the female pronucleus reached beside the swollen sperm nucleus (the male pronucleus) locating in the egg periphery. A pair of small asters connected with spindle fibers appeared between the pronuclei (Fig. 1i, i′).

At 70 min, the bright spots (the mitotic centers) moved toward the opposite poles of the associated pronuclei remained in the pheriphery. The line connecting the two bright spots was oblique to the animal-vegetal axis (Fig. 1j, j′). The nuclei and the mitotic centers migrated to the central area of the zygote and underwent the first mitosis there (Fig. 1k, k′, 1, 1′). The mitotic axis was prependicular to the animal-vegetal axis (Fig. 2d).

Discussion

The present examination shows that the sperm nucleus incorporated in the oocyte locates in the peripheral region of the zygote until meiosis is completed. The female pronucleus first migrates from the animal pole region toward the male pronucleus located in the egg periphery. The associated pronuclei move to the central area of the zygote and proceed to mitosis. Prior to the migration of the female pronucleus asters develop not near the female pronucleus, but near the male pronucleus. This suggests that the centriols derived from spermatozoa play the role(s) in nuclear migration and mitosis in oyster zygotes. It is an interesting problem whether the migration of the female pronucleus toward the male pronucleus is depended on the function of the sperm aster or its derivative, because the migration preceeds the centering of the associated nuclei.

In *Spisula solidissima*, the first mitotic spindle moves from the center to the pheriphery of the egg. The eccentric position results in unequal division at the first cleavage (Kuriyama *et al.*, 1986). In oyster zygotes, however, the mitotic apparatus stayed in the central region during first mitosis. The egg is first constricted into three lobes: two nucleated parts, equal in size, and one enucleated part (polar lobe). One of nucleated parts absorbs the polar lobe, forming a large blastomere. The difference in cleavage pattern may be related to the behavior of the mitotic apparatus.

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