

REPETITIVE PROCUREMENT OF MATURE GAMETES FROM INDIVIDUAL SEA STARS AND SEA URCHINS

JOHN W. FUSELER. From the Marine Biological Laboratory, Woods Hole, Massachusetts 02543 and the Program in Biophysical Cytology, Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

INTRODUCTION

The various conventional methods of procuring ripe gametes from sea stars (1, 2), and sea urchins (3, 4, 6) are well suited for experiments in which large quantities or pooled lots of gametes from different individuals are required.

It is often desirable, however, to perform experiments on a few cells, under well controlled conditions and from a single individual. For this purpose I have devised methods which permit repetitive procurement of healthy echinoderm eggs from a single animal for periods of a week or more. In addition, for various microscopic methods of observation and measurement, one may wish to select and use females whose eggs possess few light scattering or birefringent inclusions. The degree of optical clarity of eggs is fairly uniform for one individual, but can vary widely from one individual to another.

METHOD FOR THE SEA STAR

Asterias forbesii

The injection of sea star radial nerve extract (1), or 1-methyl adenine (1-MA) (2), into the entire animal or ovary fragments induces spawning of virtually 100% mature oocytes within 30 min. This discovery, a great improvement over earlier methods, still posed a problem. The shed oocytes could be maintained for only brief periods (1.0–1.5 h) before becoming highly susceptible to polyspermy. This "aging" could not be effectively prevented even by holding the oocytes at reduced temperatures. The method described below permits the use of one of the ten ovaries at a time from a single animal.

The ripe female is induced to autotomize a single arm containing two ovaries by making a lateral incision extending to the joint at the central disk from about midway along the arm's length. The cut is made with a scalpel (No. 12 blade) or sharp pointed scissors. Care must be exercised not to damage the ovary which lies just beneath the test. This incised arm is gently pulled, while applying slight vertical and horizontal displacements. In 2–3 min the animal releases the arm by autotomy, a probable natural escape mechanism (5). The wound

closes rapidly when the animal is returned to the seawater aquaria. No shock-induced spawning has been encountered from animals treated in this fashion.

The freed arm contains two ovaries, each attached to the aboral side of the test only by the gonoduct which is located immediately adjacent to the seam where the arm was attached to the central disk. The two ovaries must be isolated from the arm immediately to prevent damage which will otherwise occur. However, if the ovaries are removed by cutting the oviduct, shock spawning occurs in about 2 h at seawater temperature or in the cold, even when not injected with 1-MA.

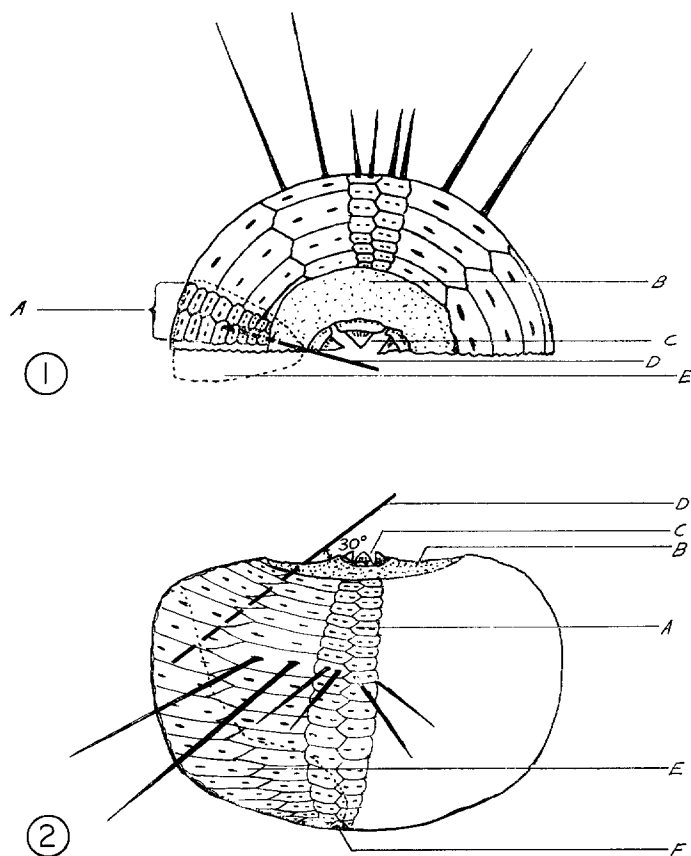
This problem is overcome by dissecting out a small (4–5 mm), square portion of the test surrounding the gonopore, keeping the oviduct intact. Each ovary can then be slid out intact from the arm by pulling on the dissected fragment of the test with forceps. Ovaries isolated in this manner can be kept for up to 14 h in filtered seawater (FSW) at seawater temperature before they shock spawn. Injection of 0.2 ml of 10^{-5} M 1-MA diluted with FSW at any time during this period yields oocytes which give 98–100% fertilization and normal development through gastrulation.

METHOD FOR THE SEA URCHIN

Lytechinus variegatus

The conventional method of injecting 0.5–1.0 ml of isotonic KCl per 50 ml of urchin body weight, which strips the animal of all ripe gametes, is unsuitable for our purposes. The application of electrical current for the induction of brief spawning was also found unsatisfactory for *Lytechinus variegatus*.

A single ovary can be induced to shed by injecting 0.2–0.3 ml of 0.53 M KCl through the peristomal membrane at its immediate junction with one of the five rows of ambulacral plates (Fig. 1). The angle of entry of the hypodermic needle should be inclined at about 30° from the oral surface of the sea urchin (Fig. 2). This localizes the small dose of KCl in or on a single ovary. The approximate position of the ovary injected with KCl is marked by clipping some of the spines on the ambulacral plates. The inverted urchin is placed over a beaker filled



FIGURES 1 and 2 Diagrammatical sketch of an inverted sea urchin illustrating the position of the hypodermic needle for KCl injection. Fig. 1, oral view. Fig. 2, side view. *A*, Ambulacral plates; *B*, peristomal membrane, *C*, mouth; *D*, hypodermic needle; *E*, approximate position of the ovary; *F*, gonopore.

to the brim with FSW or artificial seawater (ASW, MBL formula No. 4) (7). A single gonopore commences spawning mature oocytes in 2-5 min after the injection, and continues for between 15-20 min until interrupted by immersing the animal in a large volume of cold (10-12°C) seawater.

Ripe eggs yielding 98-100% fertilization were obtained routinely in this manner as frequently as three times a day, with a 3-4 h period between successive spawnings, and for up to 7 days from a single ripe female *L. variegatus*. Development of the embryo through gastrulation in all cases was normal.

We have used both of these methods extensively for routinely obtaining gametes to study the effects of temperature, pressure, and certain drugs on meiotic and mitotic spindles in individual living cells.

Supported in part by National Institutes of Health grant CA10171, National Science grant GB31739X

awarded to Dr. Shinya Inoué, and training grant 5T01HD00030.

Presented to the faculty of the University of Pennsylvania in partial fulfillment of the requirement for the degree of Doctor of Philosophy.

Received for publication 12 February 1973.

REFERENCES

- CHEAT, A. B. 1966. The Gamete-Shedding Substances of Starfish: A Physiological Study. *Am. Zool.* 6:263.
- KANATANI, H., H. SHIRAI, K. NAKANISHI, and T. KUOKAWA. 1969. Isolation and Identification of Meiosis Inducing Substance in Starfish *Asterias amurensis*. *Nature (Lond.)* 221:273.
- HARVEY, E. B. 1956. The American Arbacia and Other Sea Urchins. Princeton University Press, Princeton, New Jersey. 298.

4. TYLER, A. 1949 a. A Simple, Non-Injurious Method for Inducing Repeated Spawnings of Sea Urchins and Sand Dollars. *Collect. Net.* **19**:19.
5. DELAGE, Y., and E. HEROUARD. 1903. Les echinodermes. *Traite de zoologie concrete*. Schleicher, Paris. **III**:495.
6. COSTELLO, D. P., M. E. DAVIDSON, A. EGGER, M. H. FOX, and C. HENLEY. 1957. Methods for Obtaining and Handling Marine Eggs and Embryos. Marine Biological Laboratory, Woods Hole, Mass.
7. CAVANAUGH, G. M. 1957. Formula and Methods IV of the Marine Biological Laboratory Chemical Room. Marine Biological Laboratory, Woods Hole, Mass. 55.