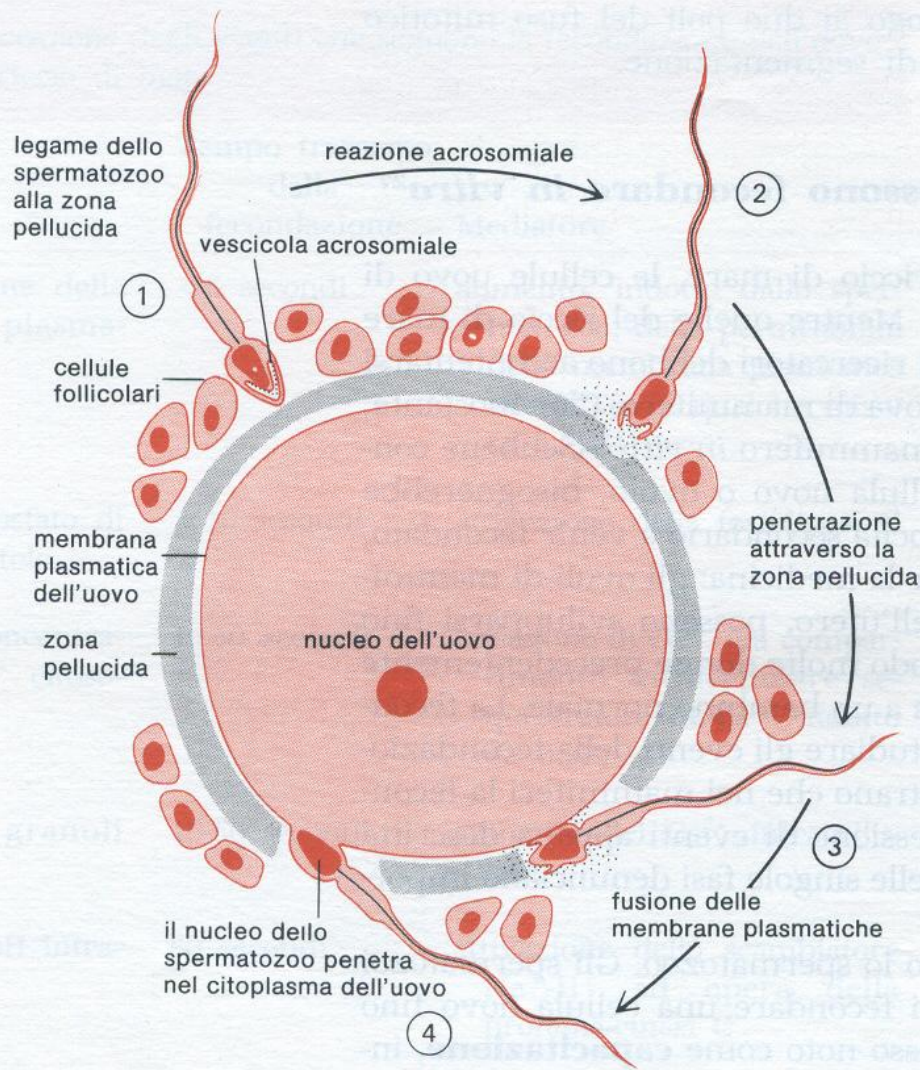
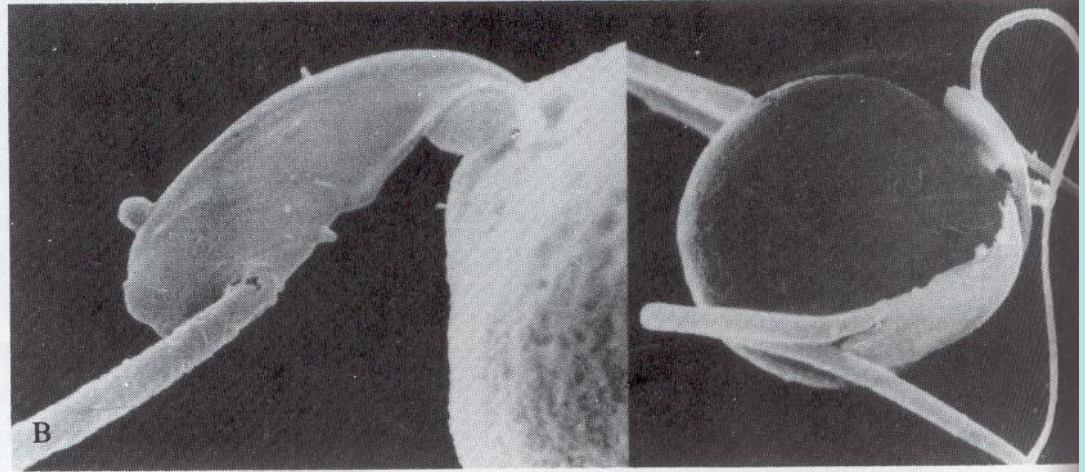
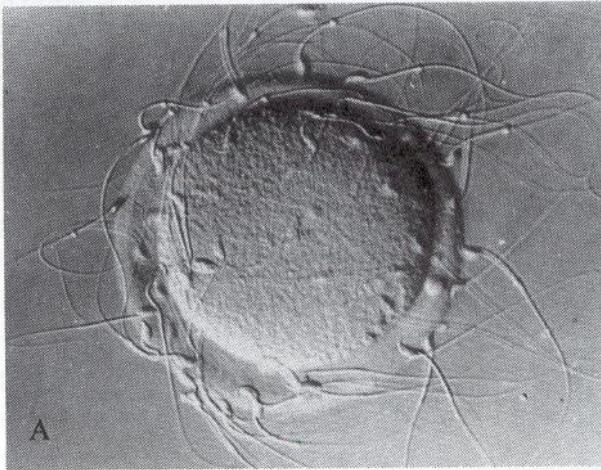


# *La Fecondazione nei pesci*

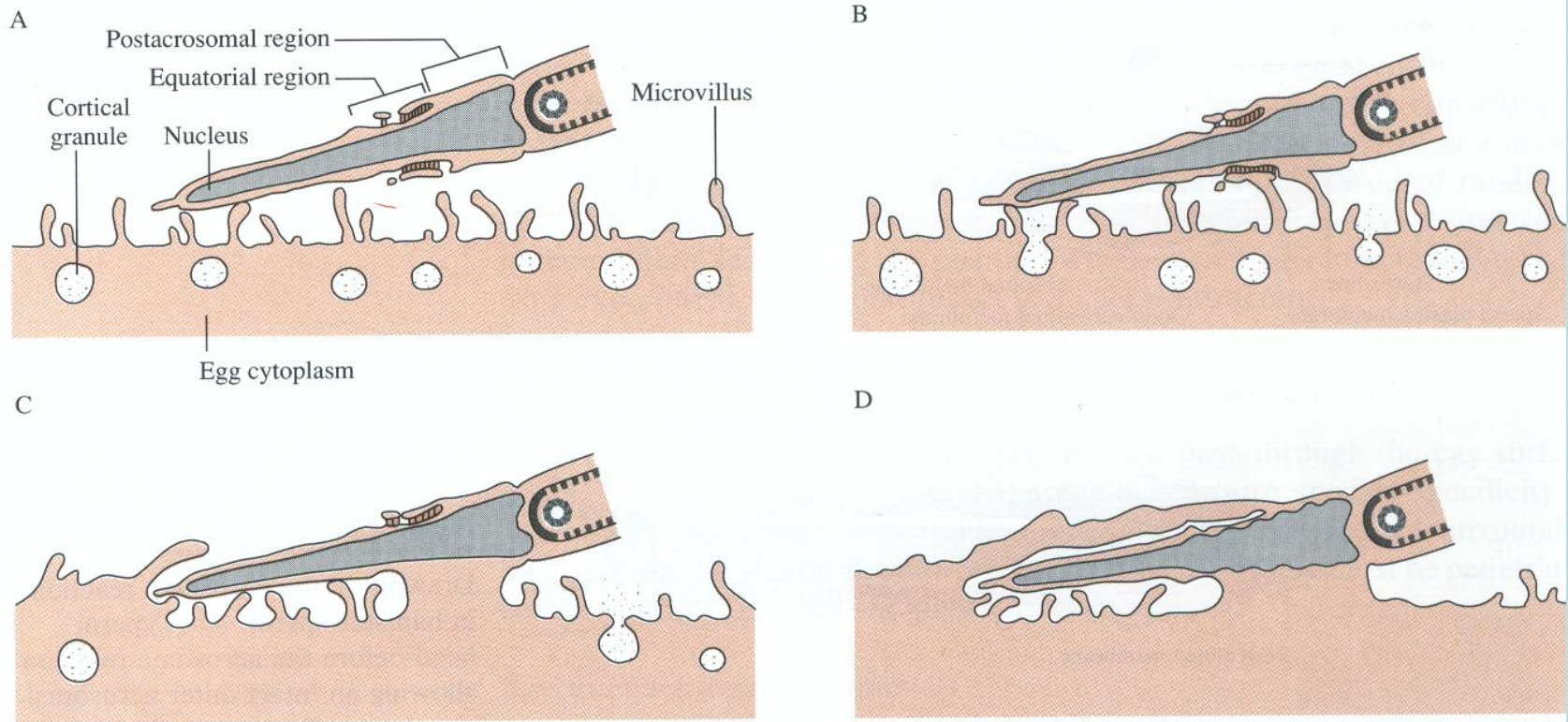


**Figura 15.49** La reazione acrosomiale che avviene quando uno spermatozoo di mammifero feconda una cellula uovo. Si suppone che nel topo un'unica proteina situata nella zona pellucida sia responsabile del legame con lo spermatozoo e, ad un tempo, dello scatenamento della reazione acrosomiale. Si noti che lo spermatozoo di mammifero interagisce con la membrana plasmatica della cellula uovo tangenzialmente, cosicché la fusione si verifica sul fianco anziché sull'apice della testa dello spermatozoo. Nel topo la zona pellucida ha un diametro di circa  $7\ \mu\text{m}$  e lo spermatozoo la attraversa ad una velocità approssimativa di  $1\ \mu\text{m}/\text{min}$ .



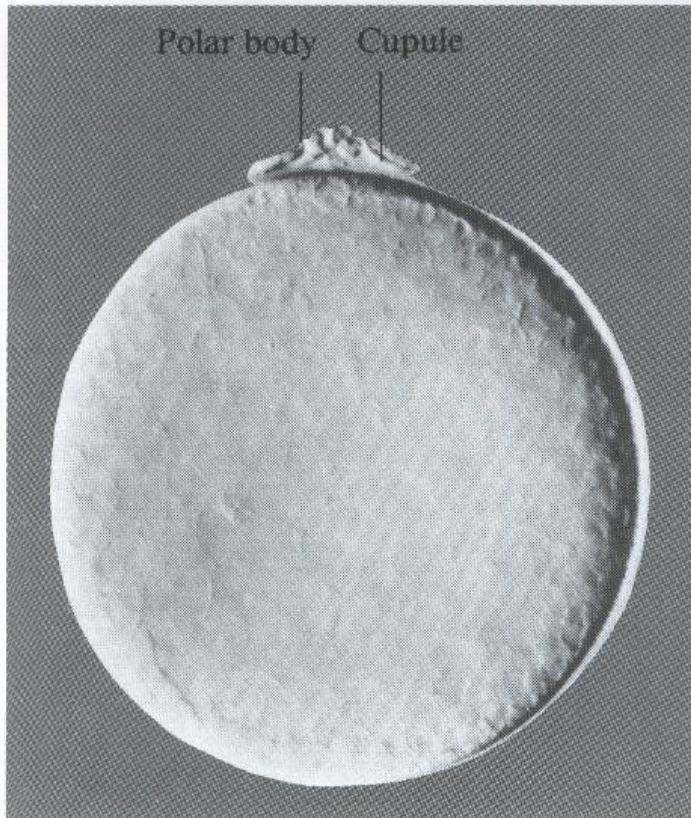
**Figure 4.8**

A, *In vitro* fertilization of a mouse egg showing many capacitated sperm binding to the zona pellucida. Only one sperm will penetrate the egg (inner sphere). B, Mouse sperm binding to a glass bead coated with the sperm receptor ZP-3. Right: entire bead with a single bound sperm. Left: close-up of sperm head bound to the surface of a glass bead. The glass bead is approximately 10  $\mu\text{m}$  in diameter. (From Wassarman, 1990. Reprinted by permission of Company of Biologists, Ltd.)



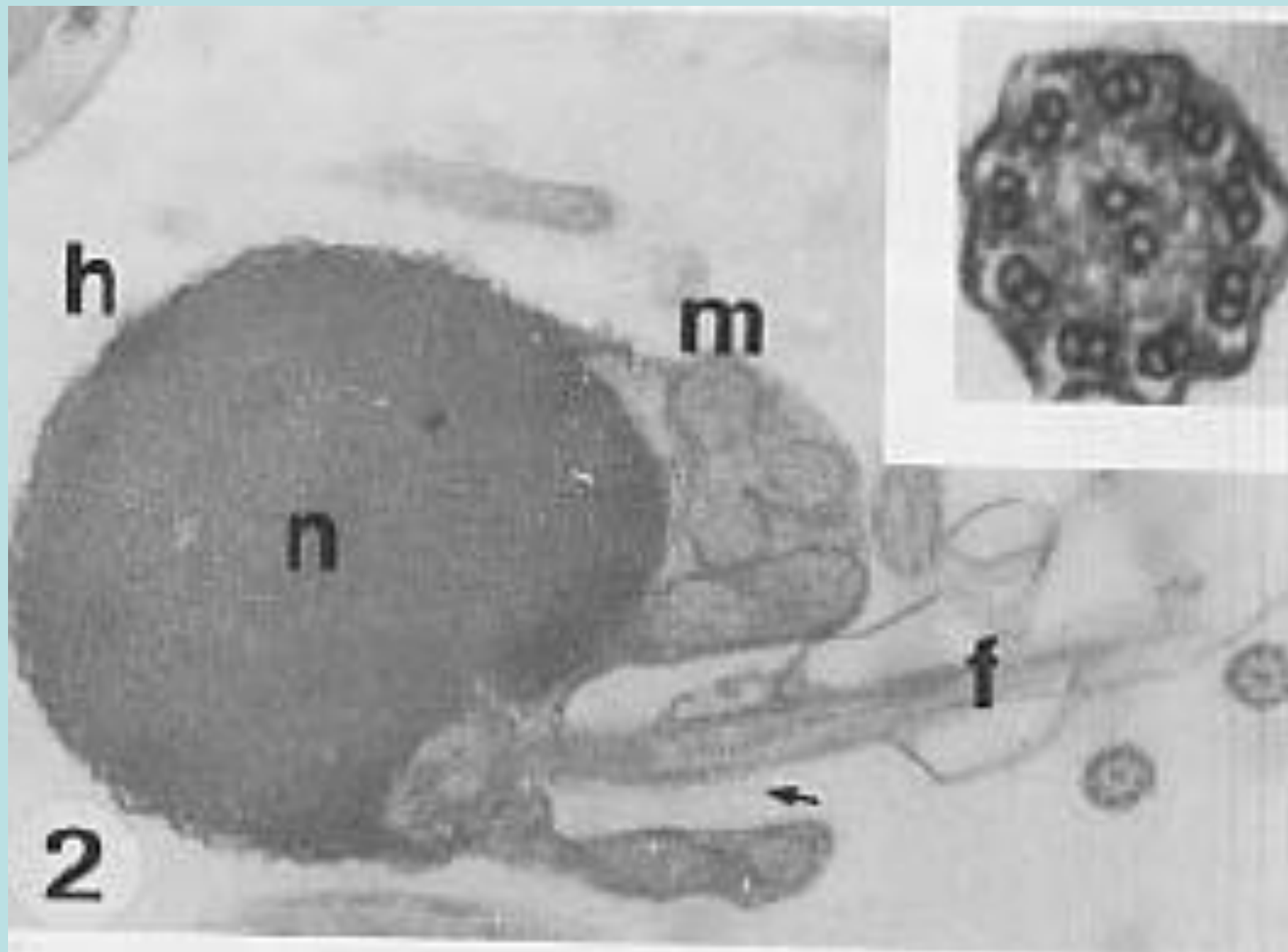
**Figure 4.7**

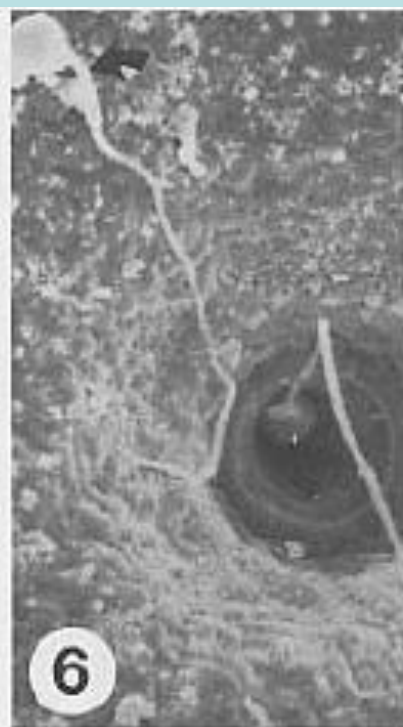
Drawings representing stages of tangential entry of sperm into the hamster egg.  
 (After Yanagimachi and Noda, 1970.)



**Figure 4.4**

Unfertilized egg of the jellyfish *Muggiaea kochi* showing the cupule, which contains the sperm attractant protein, and the polar bodies at the animal pole. (From Carre and Sardet, 1981.)





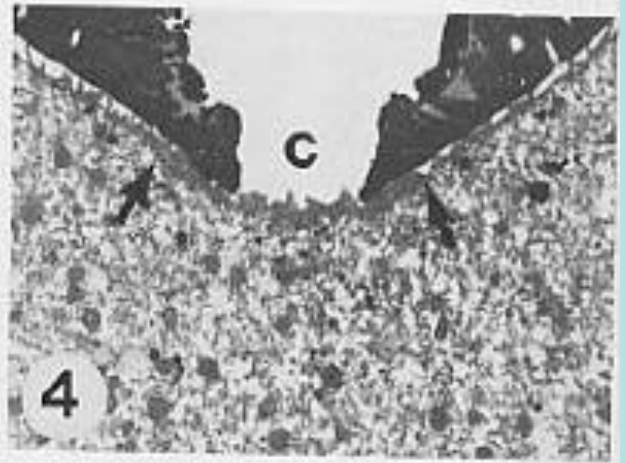
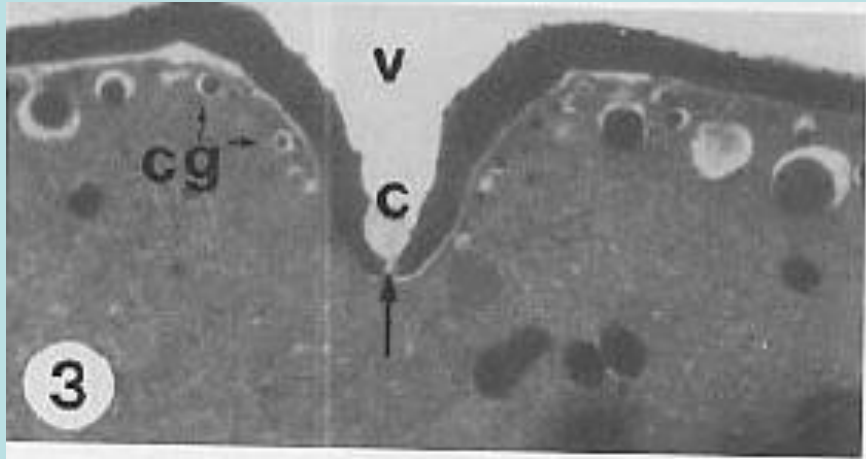
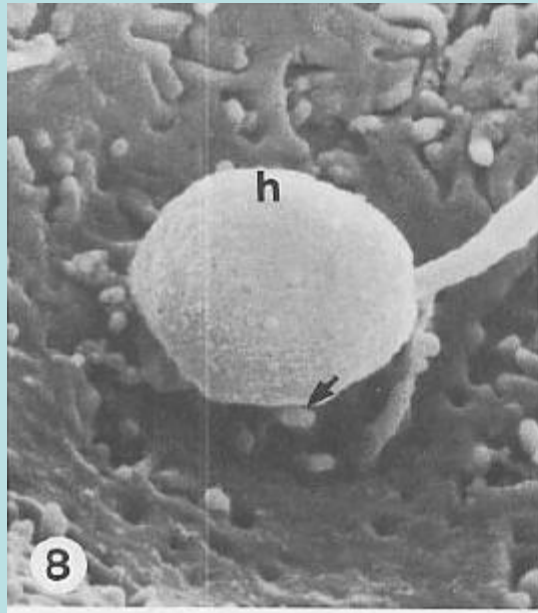
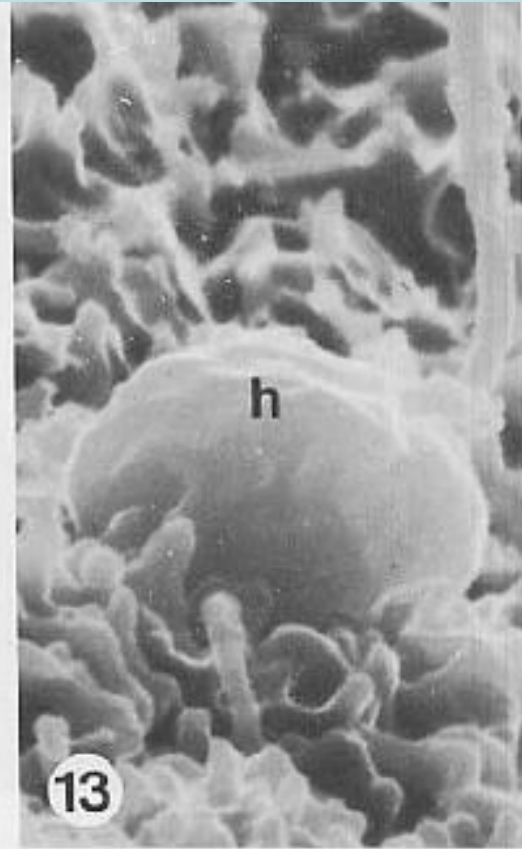
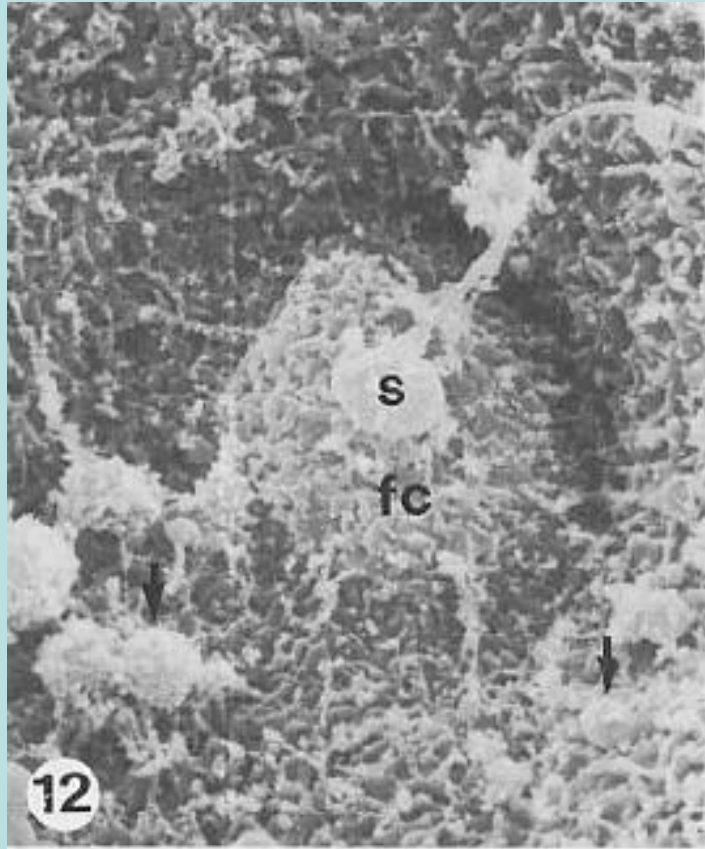
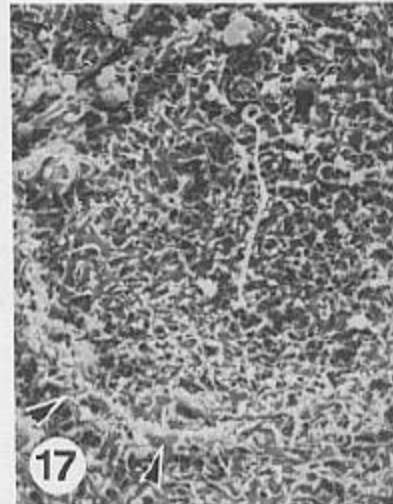
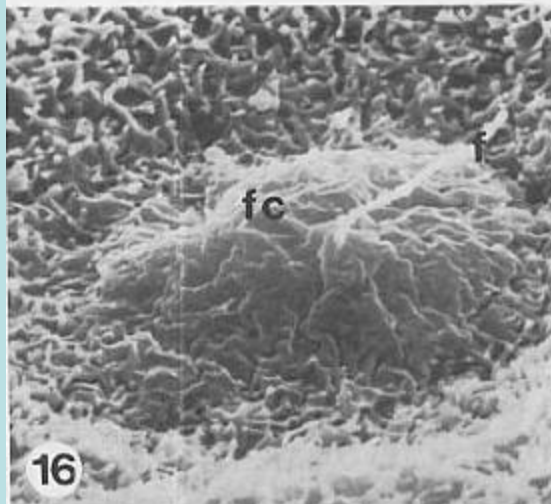
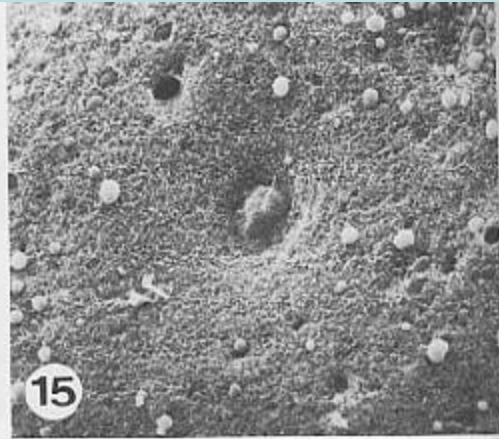
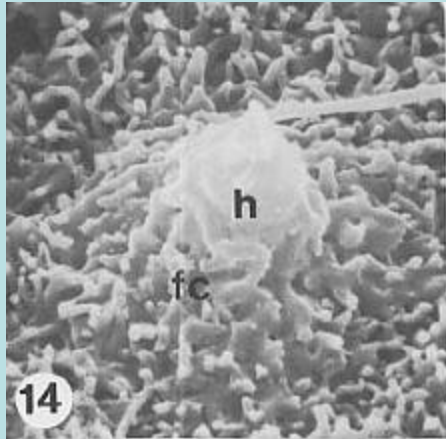
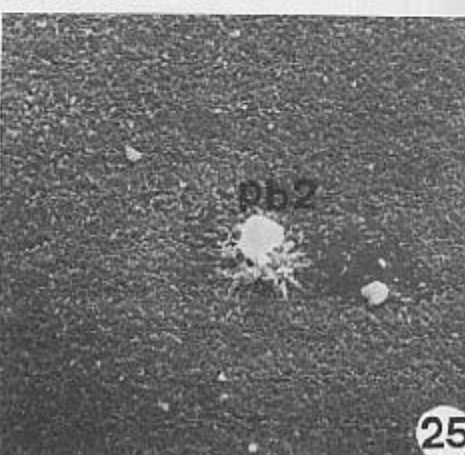
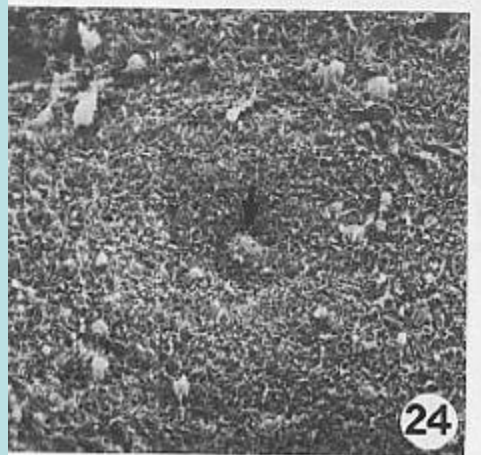
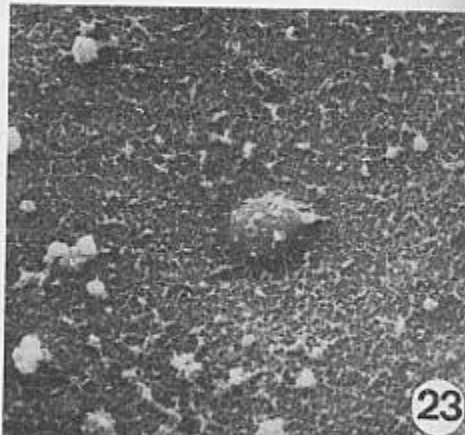
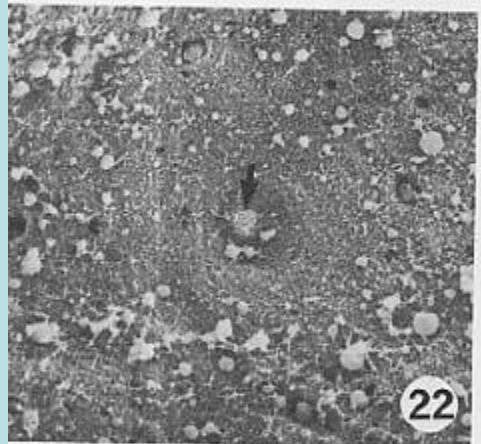
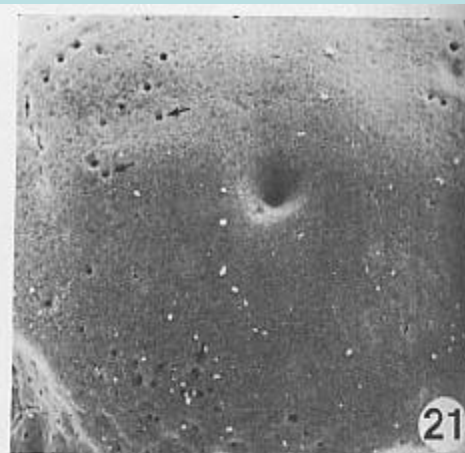
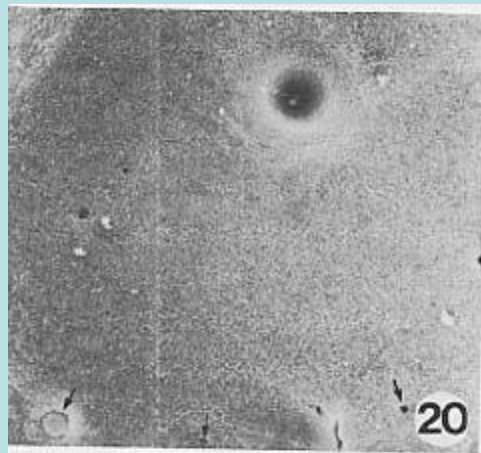


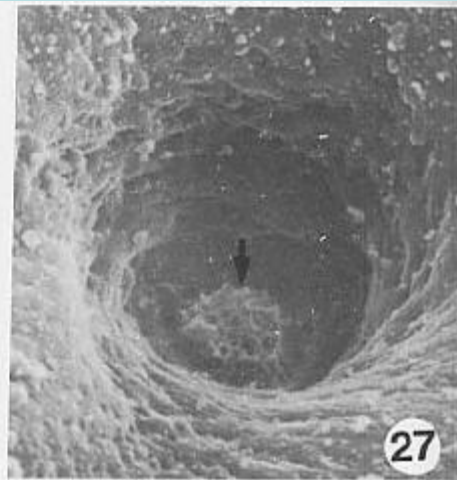
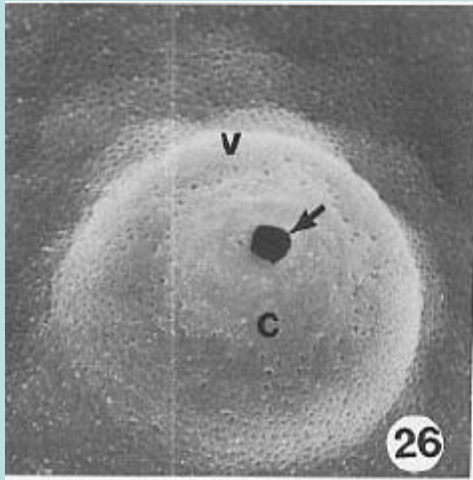
Fig. 4. (Continued)







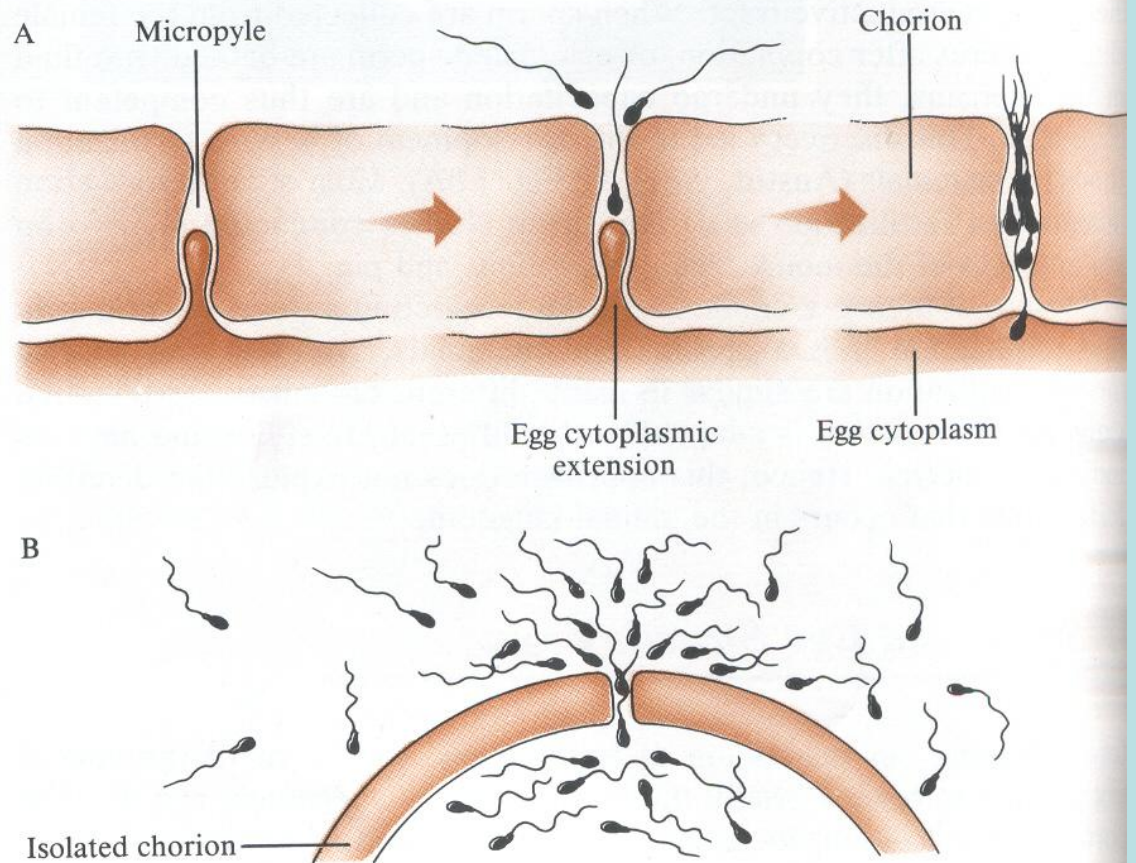




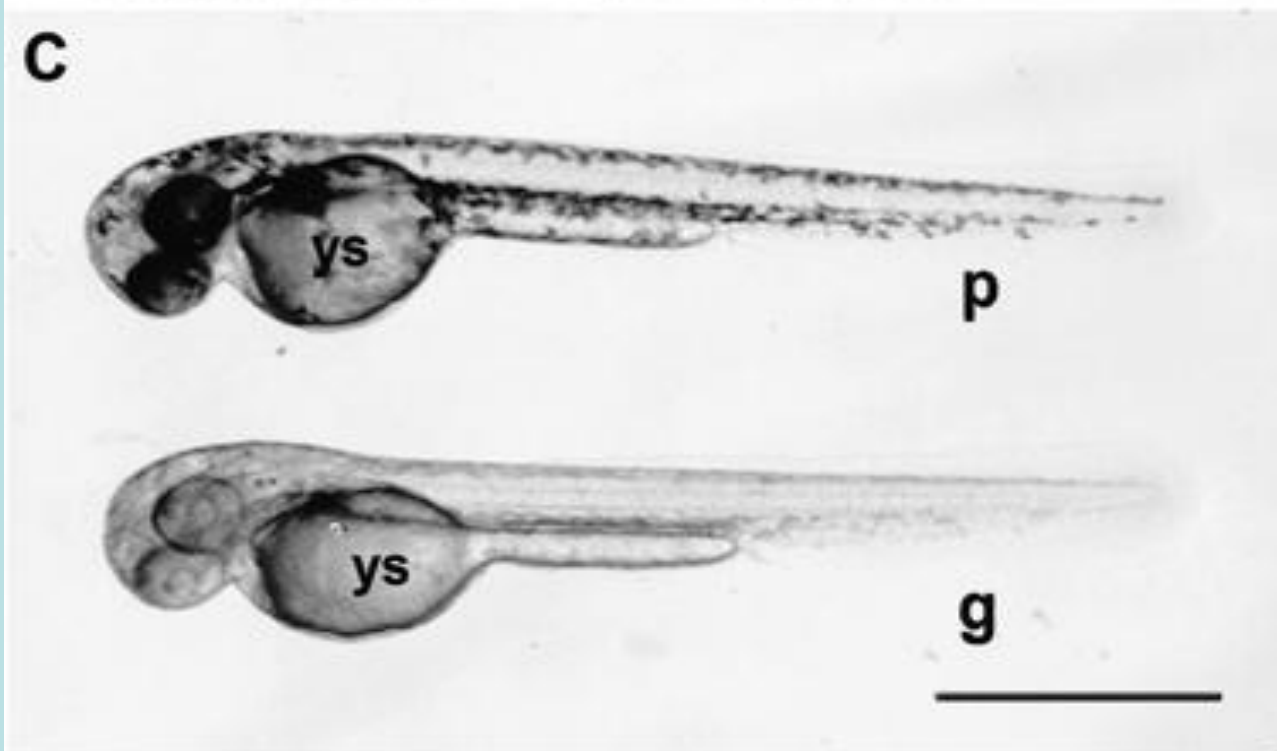
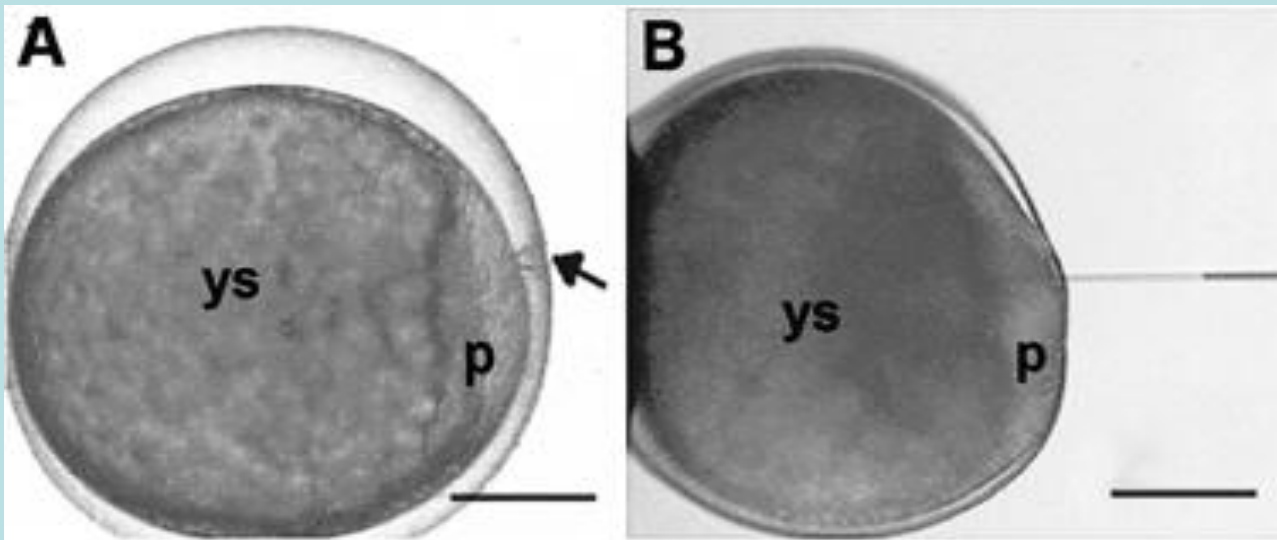
**ABSTRACT** Morphological studies on the gametes and entry of the spermatozoan into the egg of the zebra danio, *Brachydanio rerio*, were conducted primarily with scanning electron microscopy. The spermatozoan showed a spherical head, which lacked an acrosome, a midpiece containing several mitochondria, and a flagellum. Observations of the unfertilized egg confirmed and extended prior studies showing a distinct cluster of microvilli on the plasma membrane, identified as the sperm entry site, beneath the inner micro-pylar aperture (Hart and Donovan, '83). The fertilizing spermatozoan attached to the sperm entry site within 5 seconds of the mixing of a gamete suspension. Binding to the egg microvilli appeared restricted to the equatorial surface of the spermatozoan. Fusion between the plasma membranes of the interacting gametes was followed by the formation of a distinct, nipple-shaped fertilization cone. The sperm head was partially incorporated into the fertilization cone cytoplasm by 60 seconds postinsemination. The incorporation of the entire sperm head, midpiece, and a portion of the flagellum occurred between 1 and 2 minutes. During this time, the fertilization cone shortened and was transformed into a massive, blister-like cytoplasmic swelling. Concurrently, upward movements of the ooplasm resulted in the gradual disappearance of the original depression in the egg surface containing the sperm entry site. The second polar body, fully developed by 10 minutes postinsemination, formed approximately 10–15  $\mu\text{m}$  from the site of sperm penetration. Development of the fertilization cone, formation of the second polar body and exocytosis of cortical granules at the sperm entry site readily occurred in parthenogenetically activated eggs, indicating that these surface rearrangements do not require sperm binding and/or fusion.

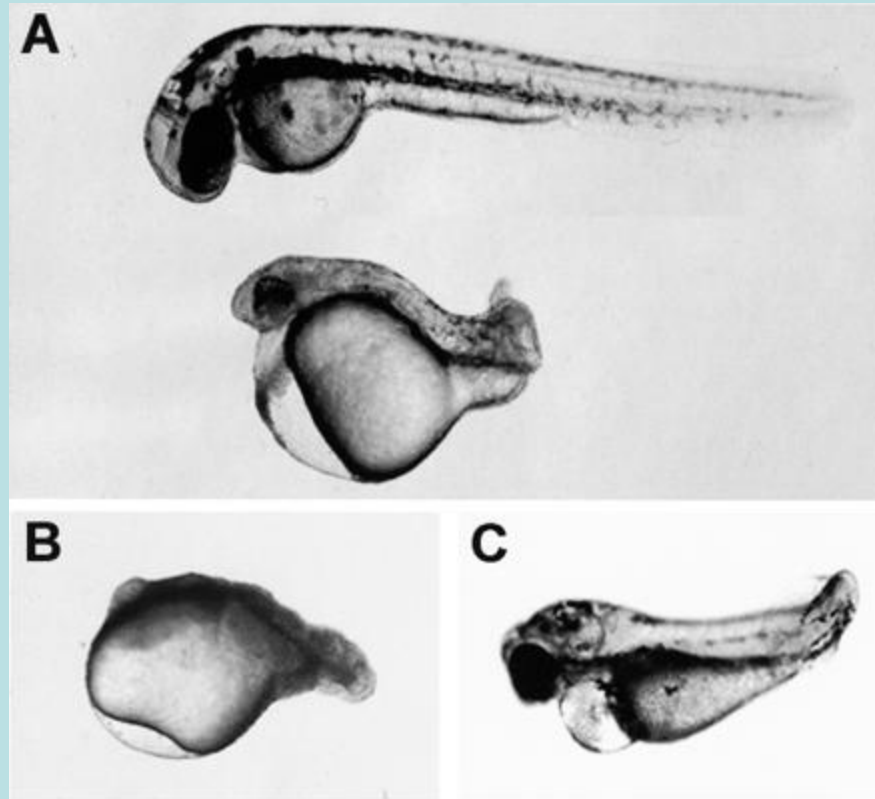
**Figure 4.1**

A, Fertilization of a sturgeon egg by a sperm that has entered the micropyle and fused with a small extension of egg cytoplasm. Although a few sperm enter the micropyle, the narrow space only permits the first to fuse with the cytoplasmic extension. Contact and attachment of the sperm to the cytoplasmic extension appears to be required for the sperm to complete its journey through the micropyle. B, Attraction of sperm to the micropyle region of the isolated chorion of a herring egg. (A, Redrawn from Austin, 1965, after Ginsberg, 1959. Reprinted by permission of Prentice-Hall Inc., Englewood Cliffs, N.J. B, After Yanagimachi, 1957.)



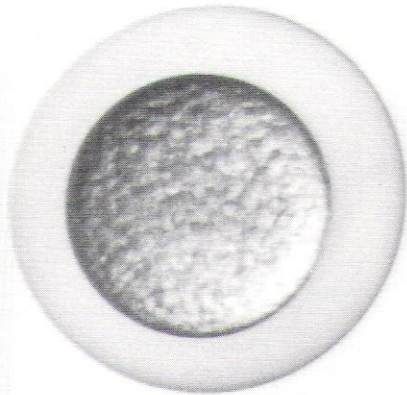
To evaluate the potential for fertilization by sperm injection into fish eggs, sperm from zebrafish, *Danio rerio*, were microinjected directly into egg cytoplasm of two different zebrafish lines. To evaluate physiological changes of gametes on the possible performance of intracytoplasmic sperm injection (ICSI), four different combinations of injection conditions were conducted using activated or nonactivated gametes. From a total of 188 zebrafish eggs injected with sperm in all treatments, 31 (16%) developed to blastula, 28 (15%) developed to gastrula, 10 (5%) developed abnormally to larval stages, and another 3 (2%) developed normally and hatched. The highest fertilization rate (blastodisc formation) was achieved by injection of activated spermatozoa into nonactivated eggs (35%). Injections were most effective when performed within the first hour after egg collection. Flow cytometric analysis of the DNA content of the developing ICSI embryos revealed diploidy, and the use of a dominant pigment marker confirmed paternal inheritance. Our study indicates that injection of a single sperm cell into the cytoplasm of zebrafish eggs allows fertilization and subsequent development of normal larvae to hatching and beyond





Embrioni di Danio rerio ottenuti con la ICSI

(a)



(b)



**Figure 19.5** Segregation of the blastodisc. (a) The newly fertilized zygote (shown within the chorion) is seemingly homogeneous. (b) Within minutes of fertilization, waves of contractile forces cause yolk-free cytoplasm to segregate to the animal pole and form the blastodisc (chorion removed and shown at higher magnification). (From Kimmel et al. 1995.)