AN ULTRASTRUCTURAL STUDY OF CROSS-FERTILIZATION

(ARBACIA ♀ × MYTILUS ♂)

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ABSTRACT

Insemination of sea urchin (Arbacia) ova with mussel (Mytilus) sperm has been accomplished by treating eggs with trypsin and suspending the gametes in seawater made alkaline with NaOH. Not all inseminated eggs undergo a cortical granule reaction. Some eggs either elevate what remains of their vitelline layer or demonstrate no cortical modification whatsoever. After its incorporation into the egg, the nucleus of Mytilus sperm undergoes changes which eventually give rise to the formation of a male pronucleus. Concomitant with these transformations, a sperm aster may develop in association with the centrioles brought into the egg with the spermatozoon. Both the male pronucleus and the sperm aster may then migrate centrad to the female pronucleus. Evidence is presented which suggests that fusion of the male pronuclei from Mytilus sperm with female pronuclei from Arbacia eggs may occur, although this was not directly observed. These results demonstrate that Mytilus sperm nuclei are able to react to conditions within Arbacia eggs and differentiate into male pronuclei.

As a rule, eggs are inseminated by sperm of their own or a closely related species. Experimental manipulation of the gametes, however, can remove barriers to cross-fertilization (10) such that ova can be inseminated with sperm from an organism of a completely different phylum. Although a considerable number of investigations employing hybrid embryos have been published (cf. references 10 and 38) few have considered the changes that the sperm nucleus undergoes upon its entry into a "foreign" cytoplasm. In the present study, results of investigations employing sea urchin (Arbacia) eggs inseminated with spermatozoa from an unrelated animal (Mytilus) are presented. The following aspects have been considered: (a) the extent to which the sperm nucleus of one animal is able to react to conditions within an egg from an

organism of a different phylum, i.e., by a comparative ultrastructural analysis of pronuclear development and association, and (b) the transformations that maternally derived organelles undergo upon insemination with heterologous sperm.

MATERIALS AND METHODS

Ripe Arbacia punctulata obtained from the Marine Biological Laboratory, Woods Hole, Massachusetts were induced to spawn according to methods outlined by Costello et al. (8). Eggs were washed in seawater and incubated in 0.05% trypsin (Sigma Chemical Co., St. Louis, Mo.) for 10 min. After washing three times in fresh seawater, the treated eggs were incubated in seawater or in seawater made alkaline (pH 8.9) according to Loeb (19). To prepare sperm suspensions, the mantles of Mytilus edulis, containing the testes, were removed, minced, and squeezed through cheese cloth into a Sten-

der dish. The sperm were kept undiluted at 4°C until used. Eggs were incubated for 15 min in a dense concentration of sperm (1 ml of undiluted sperm/10 ml of packed eggs), washed once, and recultured in seawater. (This procedure did not remove all of the sperm associated with the eggs.) For controls, sea urchin eggs, previously incubated with and without trypsin, were inseminated with homologous sperm diluted in the same manner as described above for *Mytilus*. Sea urchin eggs, which were not treated with trypsin, were also incubated with *Mytilus* sperm.

Eggs and embryos were incubated at 20°C with constant stirring at 60 rpm. Samples of the cultures were removed and prepared for light and electron microscopy at 5-min intervals for 60 min according to the methods of Longo and Anderson (22, 27). The cultures were also monitored continuously (up to 24 h) with bright field optics in order to ascertain the extent of development.

To aid in the ultrastructural visualization of the vitelline layer, ova treated with or without trypsin were incubated with ruthenium red (30, 31), in order to stain the acid mucopolysaccharide materials on gamete membranes (4, 35).

RESULTS

Effects of Trypsin Treatment

The effects of trypsin on the structure of sea urchin eggs and their ability to be fertilized with homologous or heterologous sperm have been considered by previous investigators (cf. references 10 and 37). Therefore, consideration is given only to those aspects pertinent to details considered in this communication.

Incubation of *Arbacia* eggs in trypsin decreases the thickness of the vitelline layer by 50% or more (Figs. 1 and 2). Removal of the entire layer as determined morphologically was not achieved, and such attempts usually resulted in lysis of ova. Activation of unfertilized ova by trypsin was not observed.

100% fertilization was achieved when trypsinized eggs were incubated with *Arbacia* sperm in the presence or absence of alkaline seawater (Table I). In only one experiment (one out of six) was cross-fertilization achieved when trypsinized sea urchin eggs were suspended in seawater and *Mytilus* sperm. The percentage of ova inseminated in this case was less than 5% of the total population examined. Suspensions of trypsinized sea urchin eggs and mussel sperm suspended in alkaline seawater resulted in cross-fertilization, which in these cases was highly variable (0-30% insemination) and inconsistent from one experiment to another (three out of five experiments demonstrated cross-

fertilized eggs). The highest percentage of ova cross-fertilized in any of the experiments carried out was 30%. The fixed specimens of this experiment provide the illustrative material presented in this communication.

Cross-fertilization was determined by actual observation (light or electron microscope) of spermatozoa within the cytoplasm of an egg. Changes in the cortex of sea urchin eggs were not used as criteria for cross-fertilization since all eggs that were definitely inseminated, i.e., contained at least the spermatozoon, did not demonstrate cortical alterations (see below).

Interaction of the Gametes

Mytilus sperm undergo an acrosome reaction when exposed to alkaline seawater. The acrosome reaction occurs in a manner similar to that described by Niijima and Dan (33) and results in the formation of a long (ca. 5 μm) acrosomal process (Fig. 3). Almost all of the *Mytilus* sperm that were incubated with sea urchin eggs in normal seawater possessed intact acrosomal vesicles.

Some eggs (approx. 1% of the total population) were surrounded by a dense suspension of *Mytilus* sperm (Fig. 4, *inset*), while others had only a few or none. Eggs associated with numerous sperm were invariably found to be inseminated. However, the fact that an egg did not have many sperm surrounding it was no indication that it was unfertilized.

Cross-fertilized *Arbacia* eggs may or may not initiate a cortical granule reaction. In some fertilized eggs the cortical granules remained intact (Fig. 4). Other than that these eggs revealed the presence of an incorporated spermatozoon, it was difficult to determine that they were in fact inseminated. In many eggs there was a separation of the remains of the vitelline layer from the surface of the egg, resulting in a fertilization membrane (Fig. 4, *inset*). The fertilization membrane was an uneven, fuzzy layer that was frequently incomplete. Some eggs elevated a fertilization membrane but did not demonstrate a discharge of cortical granules (cf. reference 26). In most eggs, however, particularly those surrounded by many spermatozoa, there were signs that a cortical granule reaction had taken place in the manner characteristic of untreated specimens (3).

Gamete fusion and incorporation of *Mytilus* sperm into *Arbacia* eggs were not directly observed. Images of sperm nuclei located in the
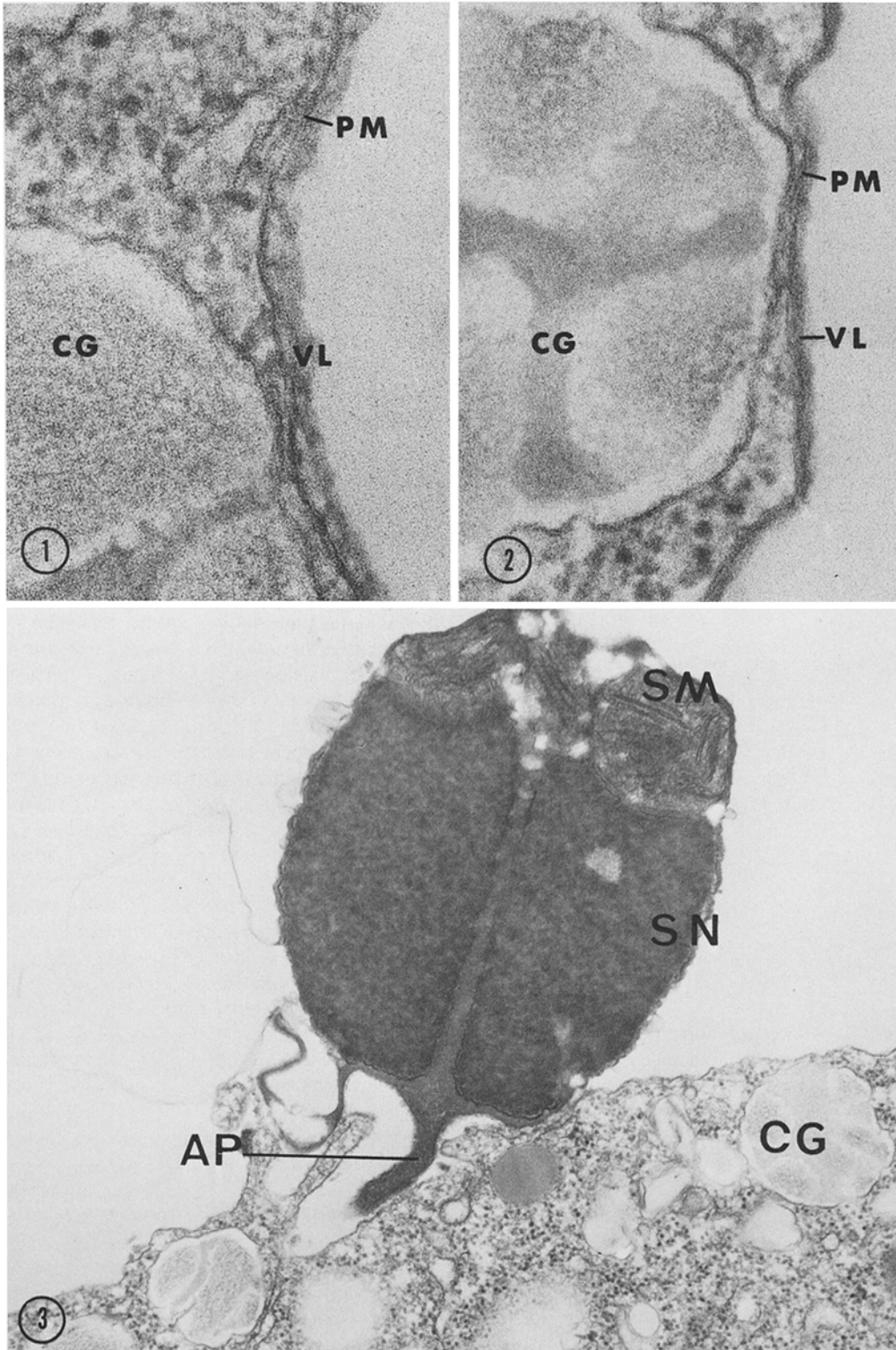
FIGURES 1-2 Unfertilized *Arbacia* eggs incubated in seawater with (Fig. 2) and without (Fig. 1) trypsin and stained with ruthenium red. The vitelline layer (*VL*) of the untreated specimen is approximately twice as wide as that of the treated specimen. *PM*, plasmalemma; *CG*, cortical granule. × 140,000.

FIGURE 3 *Mytilus* sperm that has undergone the acrosome reaction and is associated with the surface of an *Arbacia* egg. The acrosomal process (*AP*) of the spermatozoon is closely associated with the plasma membrane of the egg. *SN*, sperm nucleus; *SM*, sperm mitochondria; and *CG*, cortical granules. × 29,000.

TABLE I

Experimentally Produced Hybrids (Percentage of Eggs Fertilized) and Acrosomal Reaction with Different Treatments of Arbacia and Mytilus Gametes

Normal seawater	Acrosome reaction	Eggs fertilized
		%
Arbacia (♀) × Arbacia (♂)	+	100
Arbacia (♀) [trypsin treated] × Arbacia (♂)	+	100
Arbacia (♀) × Mytilus (♂)	−	0
Arbacia (♀) [trypsin treated] × Mytilus (♂)	−	0–4*
Alkaline seawater		
Arbacia (♀) × Arbacia (♂)	+	100
Arbacia (♀) [trypsin treated] × Arbacia (♂)	+	100
Arbacia (♀) × Mytilus (♂)	+	0
Arbacia (♀) [trypsin treated] × Mytilus (♂)	+	0–30‡

Plus and minus signs represent presence and absence of sperm having acrosomal processes, respectively.

* Only one experiment of six showed cross-fertilization.

‡ Three experiments of five showed cross-fertilization.

cortex of sea urchin eggs (Figs. 4 and 5), delimited only by a nuclear envelope, indicate that incorporation of Mytilus sperm occurs in a manner similar to that previously described for invertebrates, i.e., via the fusion of the acrosomal membrane and the plasmalemma of the egg (6). Accompanying the sperm nucleus during its entry into the cortex of the egg are the sperm mitochondria and flagellar axoneme (Figs. 4–7).

Morphogenesis of Incorporated Mytilus Sperm Nuclei

After its incorporation, the Mytilus sperm nucleus differentiates into a male pronucleus. Development of a male pronucleus is initiated with the breakdown of the sperm nuclear envelope in a manner previously described (22) (Figs. 5 and 7). As a result of the disappearance of the nuclear envelope the condensed sperm chromatin is placed in direct contact with the cytoplasm of the egg (Fig. 7) and disperses to produce a mass of diffused nucleoplasm (Figs. 6 and 7). The peripheral aspect of the dispersing condensed chromatin is distinguished by the emanation of numerous fine filaments into the dispersed nucleoplasm (Fig. 6). This pattern of chromatin dispersion is structurally similar to that which is observed during

pronuclear development in Mytilus zygotes (24).

During the latter stages of chromatin dispersion, vesicles aggregate along the periphery of the dispersed chromatin and fuse to form the nuclear envelope of the male pronucleus (Figs. 7 and 8). Unlike the male pronucleus that forms in Mytilus zygotes, that which develops in cross-fertilized Arbacia eggs is spheroidal and smaller, measuring approx. 6 μm in diameter (Table II). Nucleolus-like bodies consisting of a fine-textured material form in male pronuclei derived from Mytilus sperm incorporated into sea urchin eggs (Fig. 12). These nucleolus-like bodies are structurally similar to those observed in male pronuclei of poly-spermic Arbacia eggs (25).

During the formation of the male pronucleus, an aggregation of endoplasmic reticulum and microtubules may develop within the zygote. This aggregation may take several forms: (a) endoplasmic reticulum and microtubules may become associated with the female pronucleus to form a dense perinuclear mass (Figs. 4 and 11, insets); (b) dense aggregation of endoplasmic reticulum and microtubules may also form in association with the developing male pronucleus. This body is structurally similar to that of a sperm aster described in a number of invertebrate and mammalian zygotes (20, 21). The sperm aster of cross-fertilized Arbacia eggs has a stellate appearance; its center (centrosphere) is composed of an aggregation of endoplasmic reticulum and microtubules which surround at least one centriole (Fig. 10). (The spermatozoon of Mytilus contains two centrioles [28], and normally both are taken into Mytilus eggs upon insemination [24].) From the centrosphere emanate fascicles of microtubules (Fig. 9).

Not all male pronuclei migrate from the cortex of the zygote to the female pronucleus. Those that do may come within 5 μm of the female pronucleus. We have not observed a closer apposition of the female and male pronuclei in cross-fertilized Arbacia eggs. Many of the male and female pronuclei that come into apposition with one another, i.e., within 5 μm, are associated with a sperm aster and the perinuclear specialization of endoplasmic reticulum and microtubules described above (Fig. 11). The sperm aster and the perinuclear region of the female pronucleus may converge and become one (Fig. 11). In other cases the male and female pronuclei come into apposition with each other but they may not be associated with aggregations of microtubules and endoplasmic reticulum (Fig. 12).
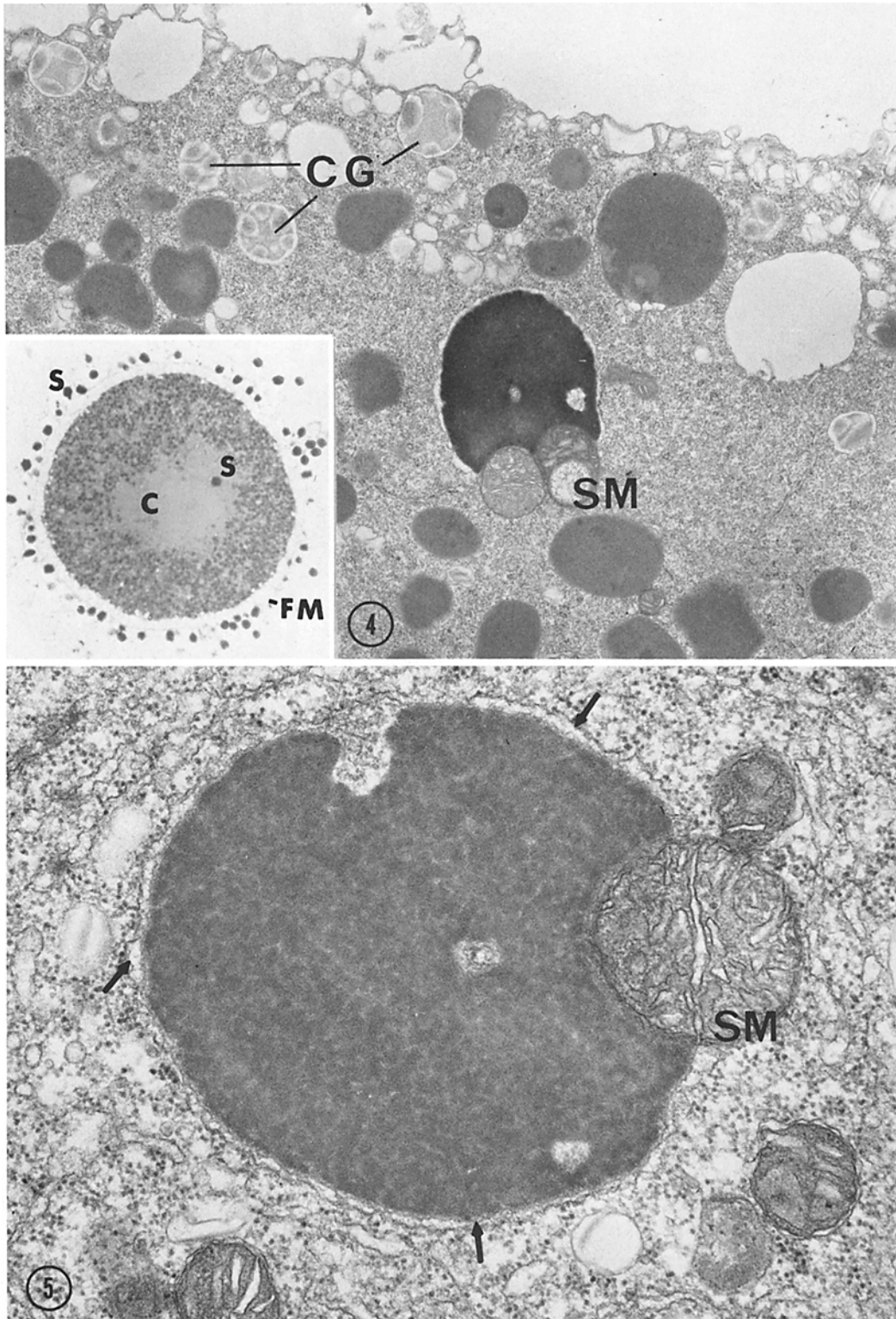
FIGURES 4–5 Incorporated *Mytilus* sperm. The incorporated spermatozoon shown in Fig. 4 has rotated (its apex is directed to the plasma membrane of the egg). Notice that this egg has not undergone the cortical granule reaction, i.e., intact cortical granules (*CG*) are observed along its surface. The sperm nucleus depicted in Fig. 5 contains condensed chromatin that is surrounded by a continuous nuclear envelope (arrows). The spermatozoon (*S*) shown in the photomicrograph is associated with a "clear" region (*C*). When examined with the electron microscope this area is seen to contain microtubules and endoplasmic reticulum which aggregate in the vicinity of the female pronucleus (see Fig. 11). Numerous sperm (*S*) are also located along the fertilization membrane (*FM*) formed by this inseminated egg. *SM*, sperm mitochondria. Fig. 4, × 12,000; Fig. 5, × 40,000; and *inset*, × 500.
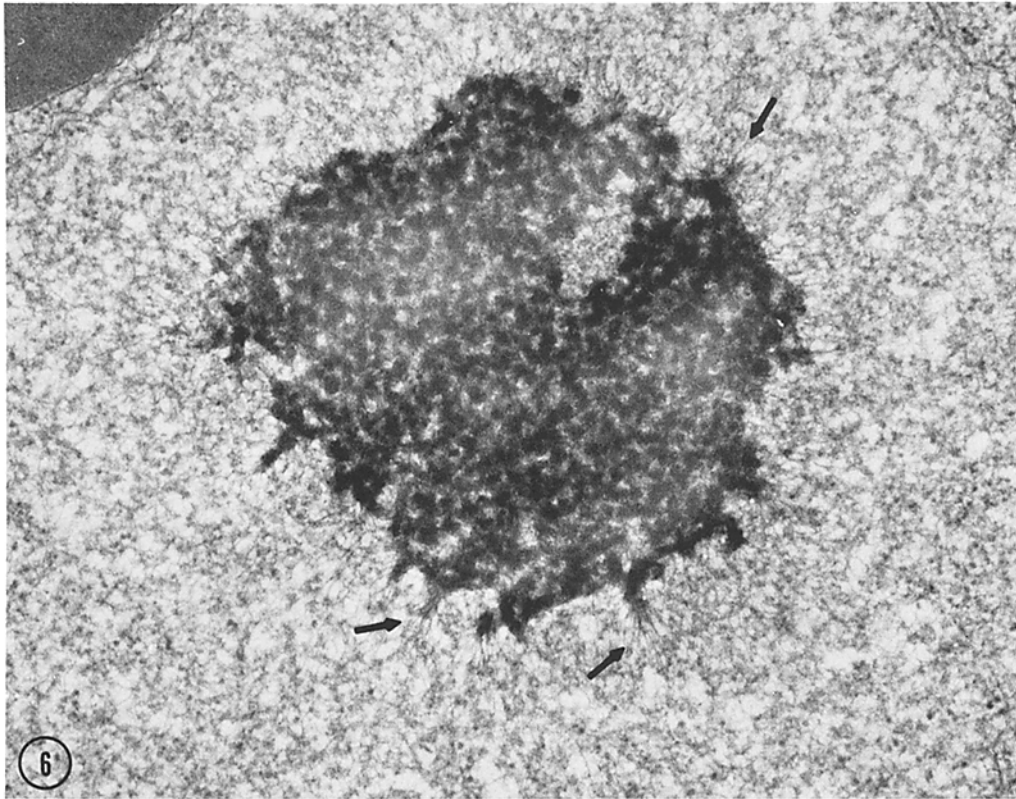
FIGURE 6 Dispersing chromatin derived from an incorporated *Mytilus* spermatozoon. Note the fine filaments (arrows) which emanate from the condensed chromatin. This pattern of chromatin dispersion is characteristic of metamorphosing *Mytilus* sperm nuclei incorporated into *Mytilus* eggs (24) and differs from that seen in male pronuclei derived from *Arbacia* sperm nuclei (22). × 40,000

We were unable to obtain direct evidence of pronuclear fusion in cross-fertilized *Arbacia* eggs. Hence, the question of whether or not pronuclear fusion occurs in *Arbacia* ♀ × *Mytilus* ♂ hybrids is unresolved. Nevertheless, some indications, admittedly not strong, were obtained which do not permit us to dismiss completely the possibility of pronuclear fusion in these hybrids: (a) Many of the female pronuclei examined 60-min postinsemination were larger than their counterparts in unfertilized or in artificially activated ova (Table II). (b) These female pronuclei also contained patches of condensed chromatin which were distributed in a manner similar to that found in zygote nuclei of polyspermic eggs (25). The condensed chromatin within the zygote nuclei of polyspermic sea urchin eggs has been shown to be of paternal origin (25). (c) Sperm axonemes were observed within the perinuclear region of female pronuclei of cross-fertilized eggs, indicating that male pronuclei may

come closer than 5 μm. Furthermore, there was no indication that the concluding events of fertilization as observed in *Mytilus* zygotes occurred in cross-fertilized *Arbacia* eggs (24).

By 2 h postinsemination most fertilized specimens contained a centrally located female pronucleus that was surrounded by an area rich in microtubules and smooth endoplasmic reticulum. Few of the eggs from any of the experiments cleaved by 24-h postinsemination (less than 1% of the eggs examined). Development of cross-fertilized *Arbacia* eggs was not monitored beyond 24-h postinsemination.

DISCUSSION

The present investigation demonstrates that treatment of *Arbacia* eggs with trypsin removes a portion of the vitelline layer and that the ability of ova to cross-fertilize is directly correlated with the removal of this egg coat. These results are in agree-
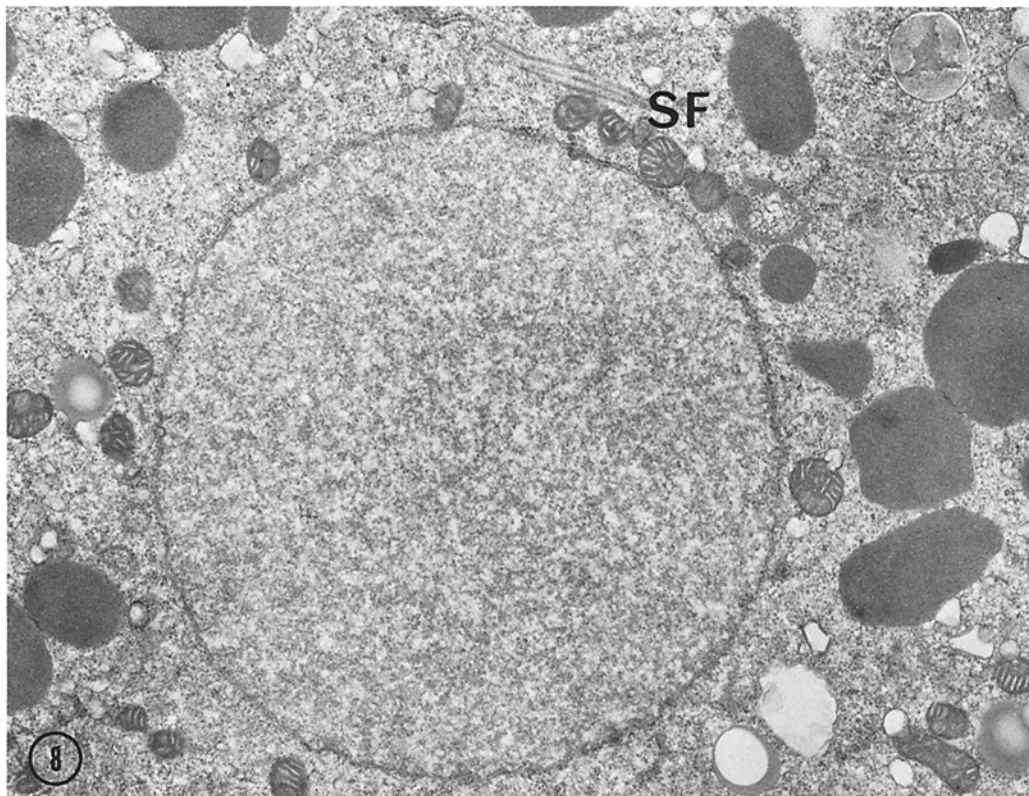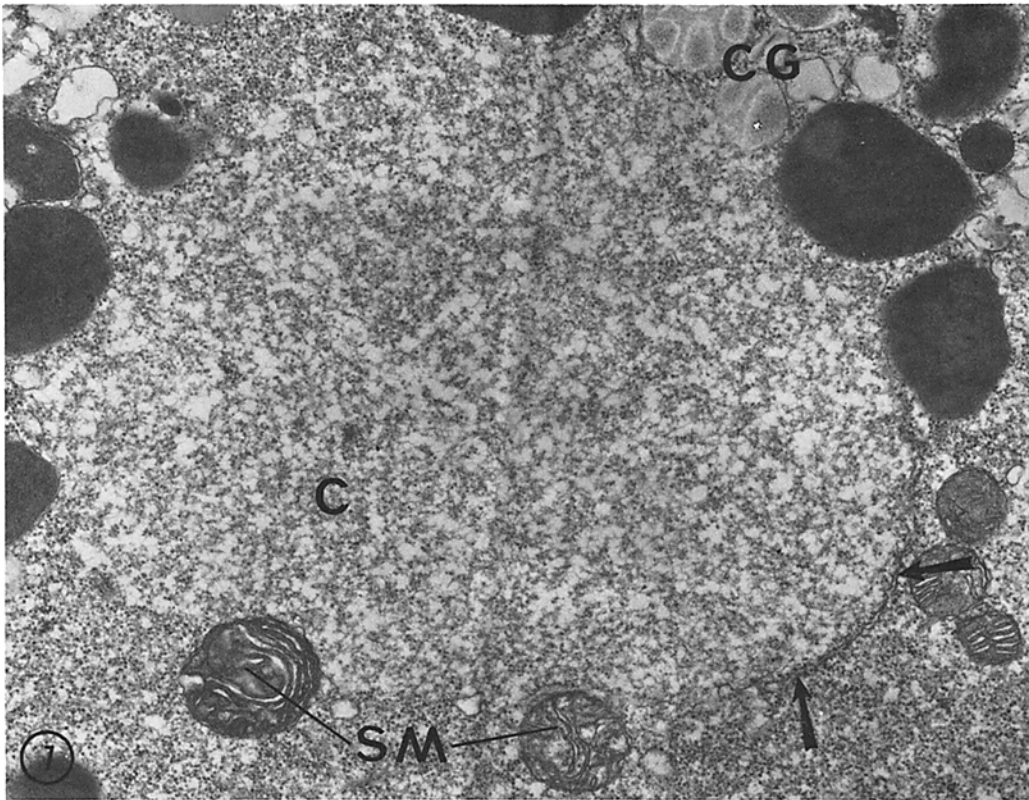
FIGURE 7   Incorporated *Mytilus* sperm nucleus which has undergone the dispersion of its condensed chromatin (*C*). Formation of the nuclear envelope that becomes part of the male pronucleus is depicted along the lower right margin of the dispersed chromatin (arrows). *CG*, cortical granules; *SM*, sperm mitochondria. × 20,000.

FIGURE 8   Male pronucleus formed as a result of the reorganization of an incorporated *Mytilus* spermatozoon. *SF*, portion of the sperm flagellum. × 15,000.

TABLE II

Diameter and Volume of Male and Female Pronuclei
in Mytilus and Arbacia Zygotes and Cross-Fertilized
Arbacia Eggs

	Nuclear diam	Nuclear vol
	μm	μm^3
Mytilus sperm nucleus	2.2 ± 0.16	5.6
Mytilus male pronucleus in Mytilus zygote	10.7 ± 1.0	647.7
Arbacia female pronucleus in Arbacia zygote	4.5 ± 0.29	48.1
Mytilus male pronucleus in Arbacia zygote	5.8 ± 0.61	103.2
Arbacia female pronucleus in fertilized egg	10.9 ± 0.67	684.7
Arbacia female pronucleus in artificially activated egg	11.1 ± 0.48	723.1
Arbacia female pronucleus in cross-fertilized egg	12.2 ± 0.67	960.1

The diameter of ellipsoidal male pronuclei in Mytilus zygotes was determined by averaging the sum of the long and short axes of specimens prepared just before pronuclear association (50-min postinsemination). Measurements of Arbacia male pronuclei were made from monospermic zygotes just before pronuclear fusion (10-min postinsemination). (Under certain circumstances, Arbacia male pronuclei are capable of becoming much larger [25, 26].) Male pronuclei of cross-fertilized eggs were measured from samples prepared 25-40 min postinsemination. Female pronuclei of artificially activated Arbacia eggs were measured from specimens engaged in DNA synthesis (56 min after activation). Measurements of female pronuclei in cross-fertilized Arbacia eggs were made from specimens which showed a perinuclear aggregation of microtubules and endoplasmic reticulum, 60-min postinsemination. Intuitively, if pronuclear fusion did occur in cross-fertilized Arbacia eggs, one might expect the volume of the female pronucleus in cross-fertilized eggs (960.1 μm^3) to equal the sum of the volumes of a Mytilus male pronucleus in Arbacia eggs (103.2 μm^3) and an Arbacia female pronucleus (684.7 or 723.1 μm^3). It does not, and this may be due to the following: (a) After pronuclear fusion, the paternally derived chromatin continues to disperse (22); this would increase the volume of the zygote nucleus over the expected sum of the volumes of the male and female pronuclei. (b) Many of the cross-fertilized Arbacia eggs were polyspermic; multiple fusions of the female pronucleus with male pronuclei derived from Mytilus sperm would result in an increase in the expected volume.

ment with similar observations of Tyler and Metz (36). Previously published studies indicate that trypsin may alter sperm-binding proteins in the vitelline layer, thereby destroying the specificity of sperm-egg binding (1, 2). The enhanced ability of trypsin-treated eggs to cross-fertilize, which has been demonstrated in the present report, supports this suggestion (cf. also references 10 and 13).

It can hardly be insignificant that the induction of an acrosomal reaction by alkaline seawater affords a means of bringing about cross-fertilization between otherwise noninteracting gametes such as Arbacia and Mytilus. Colwin and Colwin (7) claim that activation of sperm, i.e., the induction of the acrosomal reaction, is an essential prelude to gamete union (cf. also reference 9). In the invertebrates studied thus far, the acrosomal tubule is

that portion of the sperm principally involved in fusing with the egg; it is believed to possess properties which mediate the fusion of the sperm with the egg (6, 35).

The percentage of cross-fertilized Arbacia eggs reported here actually represents a minimal value since ova were considered inseminated only if sperm were located within them. Consequently, some eggs containing sperm may have been overlooked and, hence, not scored properly. Although the presence of a cortical granule reaction was not used to indicate whether or not an egg was inseminated, spermatozoa were always located in ova that underwent this early reaction to gamete fusion.

Sea urchin eggs, inseminated by sperm of an organism as evolutionarily distant as Mytilus, can undergo a cortical granule reaction. With the procedures employed during the course of this study, fertilization membranes failed to form properly after a prior treatment with trypsin. Other investigators, using different concentrations, different periods of incubation, have reported that fertilization membranes fail to form when sea urchin eggs are treated with trypsin (29, 34). The fact that sea urchin eggs undergo a cortical granule reaction indicates a "positive" early response of the ovum to gamete fusion. The presence of a cortical granule reaction and the formation of a sperm aster demonstrate that the egg is in fact activated by insemination with a heterologous spermatozoon. The reason why some eggs do not undergo a cortical granule reaction is not known. A similar heterogeneity with respect to the establishment of a cortical granule reaction has also been described in sea urchin eggs treated with urethane (26).

Since all trypsin-treated Arbacia eggs inseminated with Arbacia sperm demonstrated a cortical granule reaction, the nonuniformity of a cortical granule reaction in cross-fertilized eggs is apparently due to Mytilus sperm and/or its interaction with the egg. It is noteworthy to recall that Mytilus eggs do not undergo a cortical granule reaction upon insemination (16, 23).

Mytilus sperm incorporated into Arbacia eggs are able to initiate aster formation in a "foreign" cytoplasm. Furthermore, the centrioles brought into the egg with the spermatozoon appear to act as foci for the development of this structure. These results substantiate the long-held belief that centrioles act as an organizing center for aster formation (38; cf. reference 15).
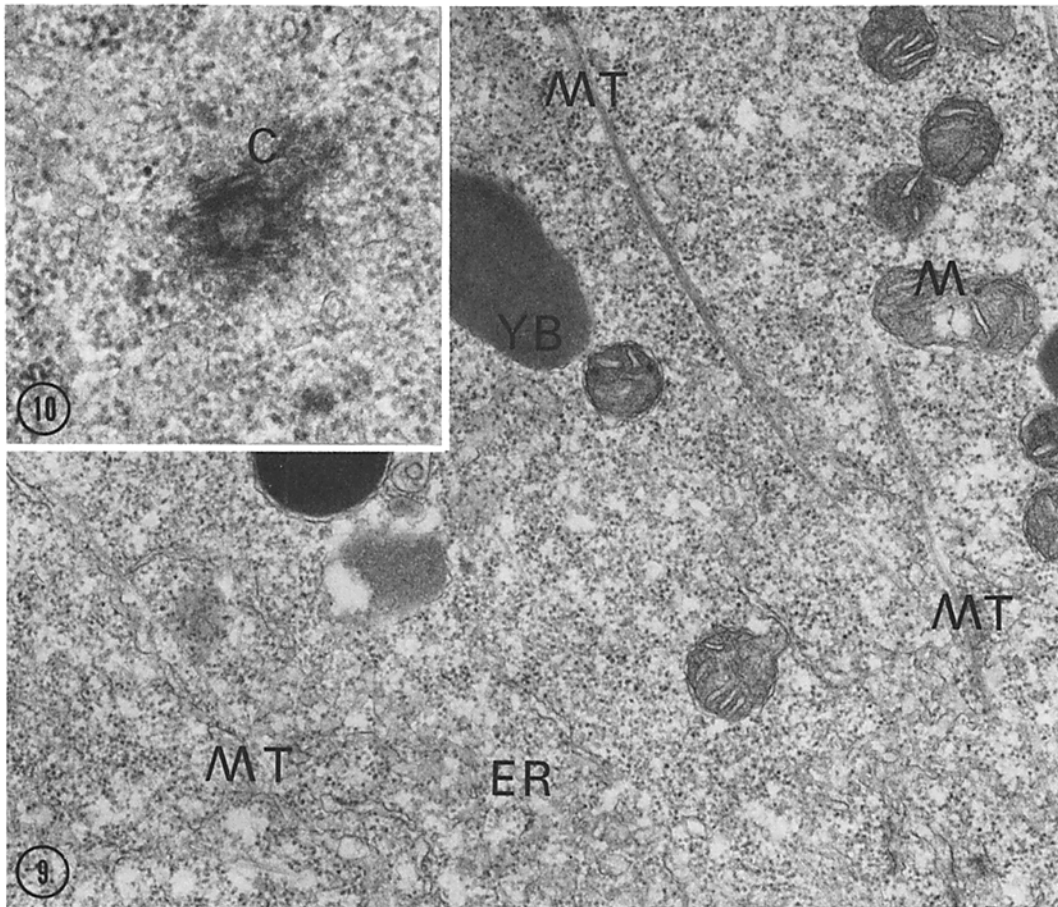
FIGURES 9-10 Peripheral (Fig. 9) and central (Fig. 10) regions of a sperm aster that developed in association with an incorporated *Mytilus* sperm nucleus. Microtubules (*MT*) and endoplasmic reticulum (*ER*) that comprise a portion of the sperm aster are shown in Fig. 9. A centriole (*C*) brought into the egg with a mussel spermatozoon is shown in Fig. 10. *YB*, yolk body; *M*, mitochondria. Fig. 9, × 30,000; Fig. 10, × 54,000.

So far as we were able to determine, most if not all *Mytilus* sperm incorporated into *Arbacia* eggs undergo chromatin dispersion and nuclear enlargement to form male pronuclei. These changes are similar to those observed in transplanted nuclei and heterokaryons (cf. references 11, 12, 14, and 17). One of the early consequences of transplantation of nuclei into *Xenopus* eggs is nuclear swelling which represents an increase of about 50 times. Nuclear swelling is thought to be related to: (*a*) The uptake of essential components (5, 32), and (*b*) the alteration of nuclear chromatin in such a manner that it can respond to whatever kind of synthesis the cytoplasm is promoting (12, 17).

The male pronuclei that develop in cross-fertilized *Arbacia* eggs contain nucleolus-like bodies

morphologically similar to those observed in male pronuclei of polyspermic *Arbacia* zygotes (25). Nucleolus-like bodies are usually not observed in the male pronuclei of *Arbacia* zygotes unless pronuclear migration or fusion is delayed (cf. references 25 and 26; F. J. Longo, personal observations). Nucleolus-like bodies have not been observed in male or female pronuclei of monospermic *Mytilus* eggs (24), and, so far as we are aware, the morphology of male pronuclei of *Mytilus* zygotes under conditions of polyspermy or delayed migration has not been reported. Consequently, the manner in which nucleolus-like bodies are formed in association with the male pronuclei of *Arbacia* ♀ × *Mytilus* ♂ hybrids requires further investigation.
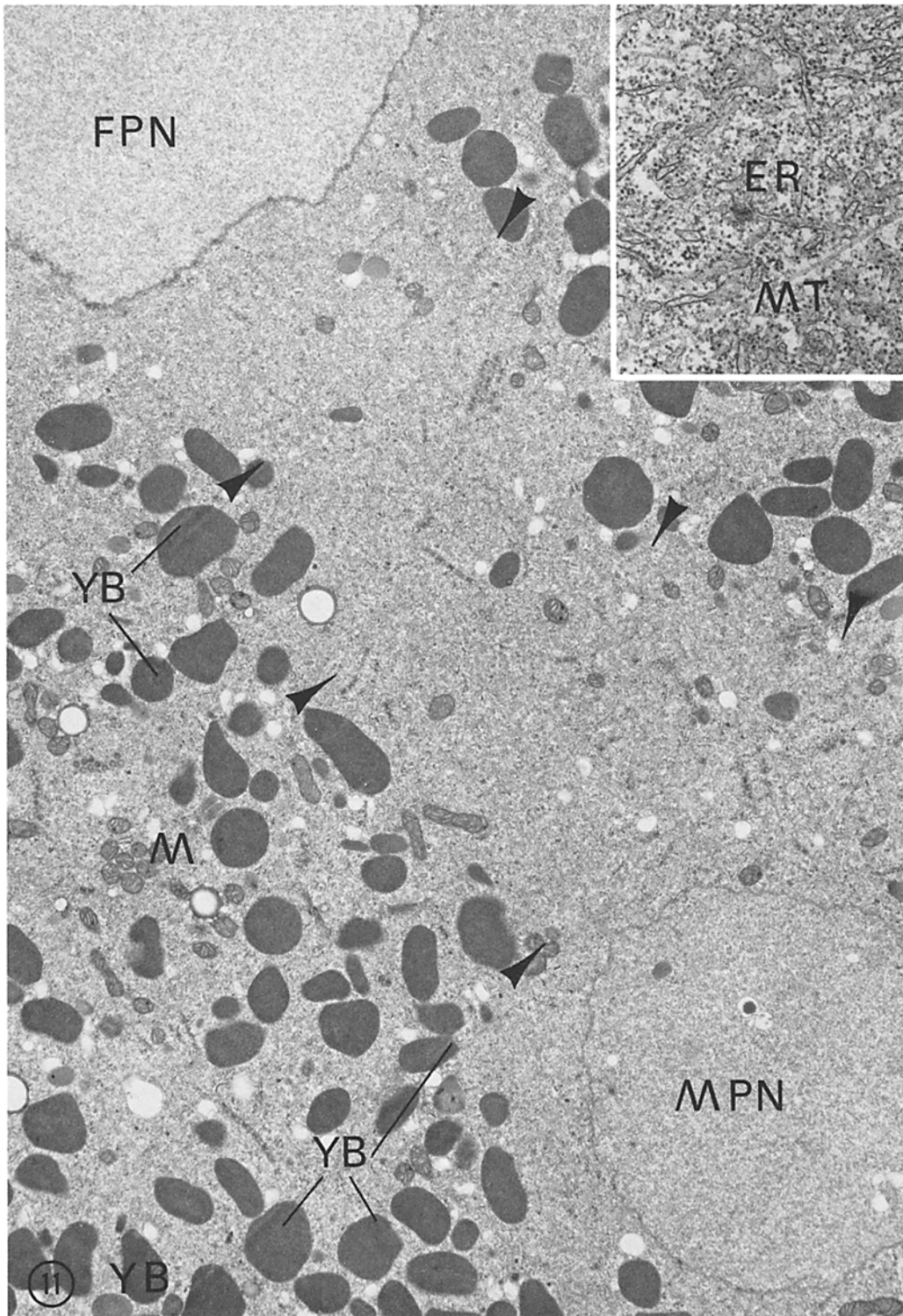
FIGURE 11   Female pronucleus (*FPN*) and male pronucleus (*MPN*) derived from an incorporated *Mytilus* spermatozoon are associated with one another by a cytoplasmic region (arrows) which is morphologically similar to that surrounding the female pronucleus (see *inset*) and rich in endoplasmic reticulum (*ER*) and microtubules (*MT*). *YB*, yolk bodies; *M*, mitochondria. Fig. 11, × 8,000; *inset*, × 32,000.
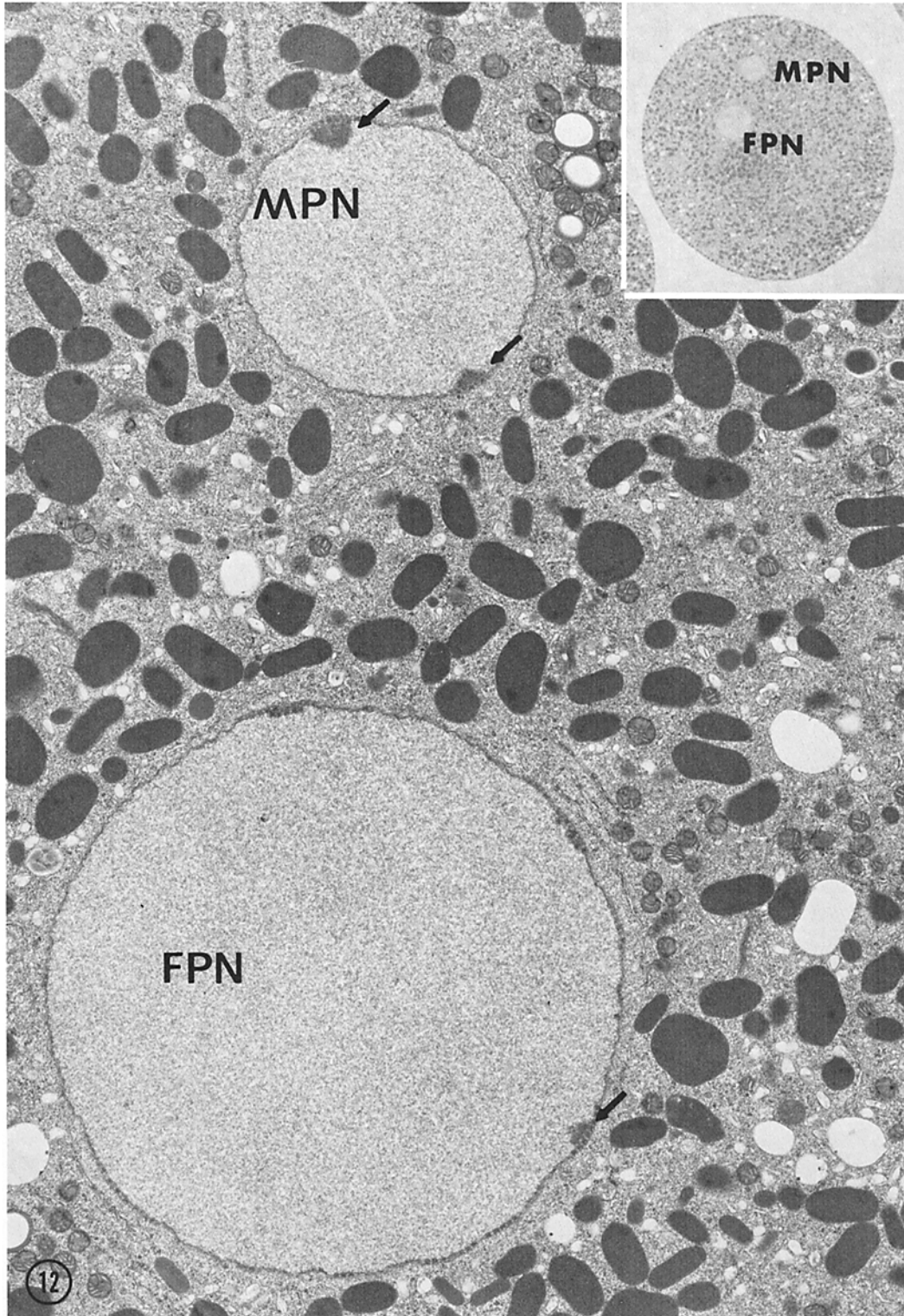
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FIGURE 12 Electron micrograph and photomicrograph of female (*FPN*) and male (*MPN*) pronuclei of cross-fertilized *Arbacia* eggs. The male pronucleus shown in the electron micrograph contains nucleolus-like bodies (arrows) which are also observed in the female pronucleus (arrow). These fine-granular structures are not found in *Mytilus* male pronuclei which develop in *Mytilus* zygotes (24). Nucleolus-like bodies are present, however, in male pronuclei derived from *Arbacia* sperm in which pronuclear migration or fusion has been delayed (25, 26). Notice that the pronuclei shown here are not associated with any specialization of the zygote cytoplasm. Fig. 12, × 8,000; *Inset*, × 500.

The male pronuclei that develop in cross-fertilized *Arbacia* eggs are similar in size to those that are derived from sea urchin sperm. The basis for this structural similarity was not established. Earlier investigations (cf. reference 38) have shown that differences in pronuclear morphogenesis exhibited by various organisms may be accounted for by the element of time, i.e., the interval between sperm entry and association of the pronuclei. If this period is prolonged in sea urchin zygotes, the male pronucleus, which is normally smaller than the female pronucleus at the time of their fusion, enlarges and the later events of fertilization take on the characteristics observed in *Mytilus* zygotes (38). In *Mytilus* the male and female pronuclei are large and equal in size at the time of their association (24). Early investigators have demonstrated (cf. reference 38) that when sperm entry occurs before the completion of polar body formation, the male and female pronuclei are approximately equal in volume at the time of their association. If, however, sperm entrance is deferred until after polar body formation the pronuclei are unequal in size, the male pronucleus being smaller than the female pronucleus.

A number of studies have indicated that factors or conditions present in eggs are involved in the metamorphoses of sperm nuclei into male pronuclei (cf. reference 20). The relation between the factors that are normally involved in the morphogenesis of homologous sperm nuclei into male pronuclei and those factors that apparently function in cross-fertilized sea urchin eggs was not determined. If such factors function in cross-fertilized eggs, they may affect pronuclear development in several ways. For example, pronuclei may develop characteristics of the species from which the spermatozoon was derived, i.e., conditions within the egg may initiate the morphogenesis of the male pronucleus but not determine its pattern (the latter being indigenous to the sperm itself). Conversely, components of the egg may act in a manner whereby male pronuclei develop characteristics of the host species. The results presented here demonstrate that *Mytilus* sperm nuclei are able to react to conditions within *Arbacia* ova and differentiate into male pronuclei. However, unequivocal evidence that pronuclear morphogenesis follows one or the other of the above proposed schemes was not obtained.

The hybrid cross used in this study has been previously investigated by Kupelwieser (18) whose observations differ from some of those presented here. Kupelwieser describes the male and female pronuclei as uniting (fusing?). No direct evidence was obtained during the course of the present study to support this earlier observation. Nevertheless, we cannot completely dismiss the possibility that pronuclear fusion may exist in *Arbacia* ♀ × *Mytilus* ♂ hybrids. The enlargement of female pronuclei in cross-fertilized *Arbacia* eggs suggests that this increase in volume may be brought about by fusion with male pronuclei derived from *Mytilus* sperm. However, this evidence is at best only circumstantial, since other explanations of nuclear enlargement in cross-fertilized *Arbacia* eggs are possible.

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