

Chapter 13

Actin Cytoskeleton and Fertilization in Starfish Eggs

Luigia Santella, Nunzia Limatola, and Jong Tai Chun

Abstract Starfish oocytes provide optimal opportunities to study meiotic progression and fertilization in vitro. A large and synchronized population of oocytes in the gonad can be induced to undergo maturation by addition of the hormone 1-methyladenine (1-MA). Successful monospermic fertilization is normally achieved when the eggs are in the interval between breakdown of the large nucleus (germinal vesicle) and extrusion of the first polar body. Insemination outside this temporal window frame gives rise to polyspermy. Although immature oocytes may become polyspermic because they are unable to form the fertilization envelope, which has been intuitively believed to serve as a mechanical block to polyspermy, overly mature eggs usually incorporate supernumerary sperm despite full elevation of the fertilization envelope. During the course of meiotic progression of the oocytes and of egg activation at fertilization, the cortical actin cytoskeleton undergoes dynamic changes. In this review, we discuss the role of the actin cytoskeleton during the meiotic maturation and at fertilization, focusing on its modulatory effects on intracellular Ca^{2+} signaling, cortical granule exocytosis, and sperm incorporation.

Keywords Actin • Ca^{2+} signaling • Ectoplasm • Polyspermy • Sea urchin • Starfish

13.1 Introduction

Oocytes of marine animals are an exceptional model system for the study of fertilization and embryonic development. Although ovulation in mammals only releases a few oocytes, starfish and sea urchin provide many eggs that are highly accessible. Fertilization and subsequent development of echinoderms can be easily observed with

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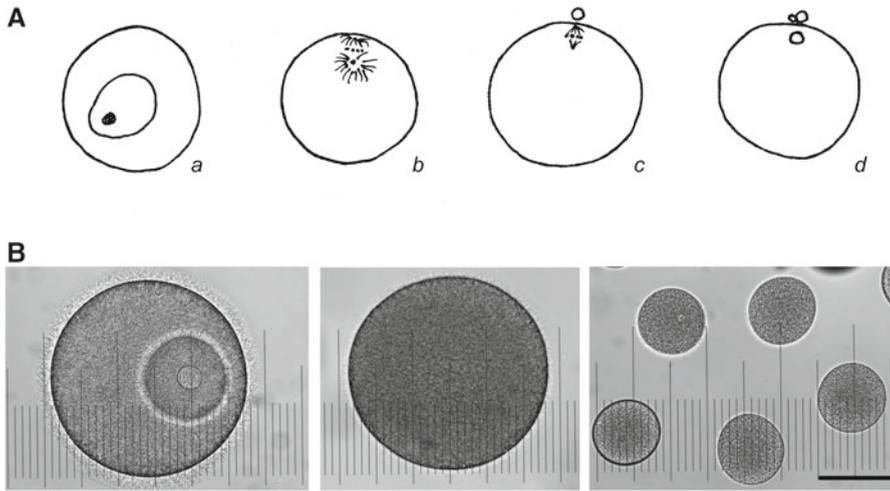


Fig. 13.1 Maturation stage and fertilization of starfish and sea urchin eggs. **(A)** Schematic diagrams of meiotic maturation stages of starfish oocytes: prophase I with large nucleus ($4n$) and nucleolus (a), metaphase I (b), metaphase II with the first polar body ($2n$) extruded (c), and completion of meiosis with female pronucleus (n) (d). Starfish and sea urchin eggs are normally fertilized at stages b and d , respectively. (Modified from Just 1939). **(B)** Micrograph of starfish and sea urchin eggs on the same scale. Bar $100\ \mu\text{m}$. *Left*, immature oocyte of *Astropecten aranciacus* at the germinal vesicle (GV) stage; *middle*, the same oocyte after germinal vesicle breakdown (GVDB); *right*, postmeiotic eggs of sea urchin (*Paracentrotus lividus*)

minimal experimental requirement, as they reproduce by “external fertilization” in seawater. At the peak of the breeding season, sea urchin ovaries are filled with eggs that have completed meiosis and contain haploid DNA (Vacquier 2011). Thus, a substantial population (<20 %) of the oocytes at the GV (germinal vesicle; i.e., the nucleus) stage can be obtained only before the peak of breeding season. At variance with the sea urchin, starfish oocytes in the female gonad at the peak of the breeding season are fully grown, yet they are arrested at prophase I of meiosis. Only upon hormonal stimulation (methyladenine, 1-MA) from the adhering follicle cells, the oocytes re-enter the meiotic cycle and are spawned into seawater (Kanatani 1973; Meijer and Guerrier 1984). Thus, even if belonging to the same phylum, sea urchin and starfish display a fundamental difference in the way in which their eggs are fertilized. Sea urchin eggs are fertilized after the completion of meiosis (haploid stage, n), but starfish eggs are normally fertilized before the completion of the first meiotic division (Fig. 13.1A). In starfish, the period between germinal vesicle breakdown (GVBD) and the extrusion of the first polar body ($2n$) represents the optimal period for successful fertilization. Indeed, insemination of the starfish oocytes before or after this period results in a high rate of polyspermic fertilization. As an experimental model, starfish provides some advantages over sea urchin eggs in that (a) meiotic maturation can be induced *in vitro*, and (b) the large cell dimensions (Fig. 13.1B) facilitate

microinjection and fluorescence imaging (Santella et al. 2008, 2012; Santella and Chun 2011). Starfish oocytes thus provide an optimal opportunity to examine the cytoplasmic changes that occur during meiotic maturation and fertilization.

13.2 Cytoplasmic Changes During Meiotic Maturation of Oocytes

13.2.1 *Morphological Transition*

During meiotic maturation, oocytes display changes in the nucleus, where chromosome reshuffling and dissolution of the nuclear membranes take place, but also morphological alteration of their surface. Before and after the maturation process, a striking structural reorganization of the cortex occurs in sea urchin eggs (Runnström 1963; Franklin 1963; Lönning 1967; Longo 1978). Transmission electron microscopy (TEM) has revealed that numerous long microvilli that are present on the surface of immature oocytes nearly disappear in mature eggs (Dale and Santella 1985). These microvilli are filled with actin filaments (Tilney and Jaffe 1980). The cortex of starfish oocytes also undergoes mechanical and morphological changes during meiotic maturation. The microvilli become shorter, and the cortical granules are translocated and positioned perpendicularly to the plasma membrane (Hirai and Shida 1979; Longo et al. 1995; Santella et al. 1999). Change of the actin cytoskeleton is often shown by the transient formation of a F-actin spike a few minutes after 1-MA addition (Schroeder 1981; Schroeder and Stricker 1983), whereas the rigidity of the cell and its F-actin content decrease at the time of GVDB (Shôji et al. 1978; Heil-Chapdelaine and Otto 1996). Thus, it appears that the actin cytoskeleton is regulated differently, presumably playing different roles, in different subcellular domains. Because the cell nucleus is enriched with actin molecules that normally do not form filaments (Clark and Merriam 1977), the role of the “spilling” actin at the time of GVDB, when the nucleoplasm inevitably intermixes with cytoplasm, is of obvious interest.

13.2.2 *Signaling Pathways to Meiotic Maturation*

The starfish oocyte maturation hormone (1-MA) operates through a receptor on the plasma membrane because microinjected 1-MA does not induce oocyte maturation (Kanatani and Hiramoto 1970; Shida and Hirai 1978). The identity of this receptor has not been determined as yet, but it is known that its activation leads to the release of G β proteins (Jaffe et al. 1993; Chiba et al. 1995), which in turn activate a phosphatidylinositol-3-kinase (PI3K)-producing phosphatidylinositol-3,4,5-trisphosphate (PIP3) from phosphatidylinositol-4,5-bisphosphate (PIP2).

An important target of the PI3K signaling pathway is the Akt/PKB kinase, which directly downregulates Myt1 and upregulates Cdc25, leading to the activation of the universal cell-cycle regulator, the cyclin B-Cdc2 complex (Masui 2001; Prigent and Hunt 2004; Kishimoto 2011).

13.2.3 Intracellular Ca^{2+} Increase During Meiotic Maturation

Artificial elevation of intracellular Ca^{2+} level can induce nuclear maturation of starfish oocytes, suggesting that Ca^{2+} is the functional downstream effector of 1-MA (Moreau et al. 1978). Nonetheless, the maturation of starfish oocytes can proceed without detectable Ca^{2+} transients or in the presence of Ca^{2+} chelators (Witchel and Steinhardt 1990), suggesting that the breakdown of the nuclear envelope and meiotic resumption may not be under the control of Ca^{2+} . However, direct microinjection of the Ca^{2+} chelator BAPTA into the GV blocked the disassembly of the nuclear envelope (Santella and Kyojuka 1994; Santella et al. 1998), suggesting that Ca^{2+} signal in the GV subcellular domain may be important. A more recent investigation has shown that the 1-MA-triggered Ca^{2+} wave always initiates in the vegetal hemisphere of the starfish oocyte and propagates to the animal pole through the superficial domains of the cortex, raising the question of the involvement of the Ca^{2+} -releasing pathway linked to the 1,4,5-inositol trisphosphate ($InsP_3$) receptors, as the latter are known to be more sensitive in the animal pole of the egg (Lim et al. 2003; Kyojuka et al. 2008). Because 1-MA can induce the Ca^{2+} response in the absence of external Ca^{2+} , the involvement of nicotinic acid adenine dinucleotide phosphate (NAADP) in the process also becomes questionable, as NAADP is known to act mainly by inducing Ca^{2+} influx in starfish oocytes.

13.2.4 Sensitization of the Ca^{2+} -Releasing Mechanisms

Starfish oocytes undergoing meiotic maturation become progressively more sensitive to $InsP_3$. Thus, mature eggs respond to the same dose of $InsP_3$ with a much higher level of Ca^{2+} release (Chiba et al. 1990; Lim et al. 2003). This response might be partly explained by the observation that the endoplasmic reticulum (ER), which constitutes the major intracellular Ca^{2+} store of the oocyte, undergoes extensive restructuring during meiotic maturation (Stricker 2006). The morphological changes of the ER parallel the alteration of the actin cytoskeleton, which readily responds to 1-MA and forms thick bundles that align perpendicularly to the egg surface 50 min after the addition of the activating hormone (Santella et al. 2008; Santella and Chun 2011). Because the sensitization of the $InsP_3$ -dependent Ca^{2+} -releasing mechanism induced by 1-MA is blocked by agents promoting actin depolymerization, that is,

latrunculin-A (LAT-A), it appears that changes of the actin cytoskeleton have a role in optimizing the Ca^{2+} -releasing mechanism in maturing oocytes (Lim et al. 2002). The actin cytoskeleton may contribute to the process, either by assisting the mobilization of Ca^{2+} from the ER or by changing the microdomain environment of the ion channels. We have provided support for this idea by demonstrating that the magnitude, kinetics, or onset of Ca^{2+} signaling in response to the fertilizing sperm, or to Ca^{2+} -linked second messengers such as InsP_3 and cyclic ADP ribose (cADPr), are significantly modified by interfering with the dynamic changes of the actin cytoskeleton: that is, with the actin-binding protein cofilin (Nusco et al. 2006), LAT-A, with Jaspilakinolide, heparin (Puppo et al. 2008), or the PIP2-sequestering domain of PLC- δ 1 (Chun et al. 2010). Interestingly, the exposure of the mature eggs of some starfish species, such as *Astropecten aranciacus*, to these treatments triggers an auto-catalyzing Ca^{2+} wave in the absence of fertilizing sperms or of Ca^{2+} -mobilizing second messengers. The Ca^{2+} wave induced by LAT-A originates largely from the intracellular stores, and its spatiotemporal pattern in Ca^{2+} -free seawater is highly reminiscent of the fertilization Ca^{2+} wave induced by sperm, although it requires a latent period of 6–10 min (Lim et al. 2002). This effect was not observed in the GV-stage oocytes, suggesting that the threshold for the triggering of the spontaneous Ca^{2+} release is much lower in mature eggs, and that the trigger itself is dependent on the actin cytoskeleton. Alternatively, Ca^{2+} may be released during the process of actin treadmilling (Lange and Gartzke 2006), and it may simply reflect a slower turnover rate of the actin filaments in immature oocytes. In other words, F-actin may contribute to intracellular Ca^{2+} homeostasis and ion flux either by modulating the ion channel activities, or by a more direct mechanism in which the polymerization and depolymerization cycle of actin filaments in the local environment may sequester and release cytosolic Ca^{2+} (Lange and Gartzke 2006; Chun and Santella 2009).

13.2.5 Changes of the Electrical Property of the Plasma Membrane During Meiotic Maturation

The aforementioned changes of cytoplasm are accompanied by decrease of oocyte stiffness within 7–8 min after 1-MA addition and by the decrease in K^+ conductance that leads to depolarization of the membrane potential (Miyazaki et al. 1975; Miyazaki and Hirai 1979). An abrupt switching of the membrane potential from the initial level of -70 to -90 mV to a new stable state of -10 to -20 mV takes place concurrently with, or shortly before, GVBD in the maturing oocytes of *A. aranciacus* (Dale et al. 1979). Thus, the resting potential of mature eggs is far less negative than that of the immature oocytes. However, the membrane potential can be restored to the original level of the GV-stage oocytes as the eggs become overripe in seawater (Miyazaki et al. 1975; Miyazaki and Hirai 1979).

13.3 Signals of Fertilization and Egg Activation

13.3.1 *Generation and Propagation of the Intracellular Ca^{2+} Wave*

Mature eggs are metabolically repressed, but interaction with the fertilizing sperm triggers a series of events that are collectively termed “egg activation.” The two earliest detectable changes at fertilization are rapid depolarization of the egg plasma membrane and Ca^{2+} influx, which are followed by the intracellular Ca^{2+} wave propagating from the site of successful sperm–egg interaction (Steinhardt et al. 1971; Shen and Buck 1993). It has been shown that intracellular Ca^{2+} increase at fertilization can be recapitulated by the combined effect of Ca^{2+} -mobilizing second messengers, namely, InsP_3 , NAADP, and cADPr. InsP_3 is generated by the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP2) in the plasma membrane by phospholipase C. Several isozymes that catalyze this process have been identified, and fertilization is thought to activate at least one of them. In analogy to the InsP_3 receptor (Streb et al. 1983; Furuichi et al. 1989), cADPr may bind to the cADPr-sensitive ryanodine receptor complex and release Ca^{2+} from the internal stores (Carafoli et al. 2001; Lee 2002; Santella and Chun 2013). The Ca^{2+} -releasing activities of both InsP_3 and ryanodine receptors are facilitated by Ca^{2+} itself through a “ Ca^{2+} -induced Ca^{2+} release” (CICR) mechanism, whereas the activity of the channels responding to NAADP does not display direct CICR properties and may involve a distinct intracellular (or extracellular) Ca^{2+} store (Santella et al. 2000; Moccia et al. 2003; Galione and Churchill 2002). At variance with the findings in the sea urchin eggs where NAADP releases Ca^{2+} from lysosome-like acidic stores (Churchill et al. 2002), the Ca^{2+} increase induced by microinjected NAADP in starfish eggs appear to require external Ca^{2+} and is mediated by the activation of ion channels on the plasma membrane (Runft et al. 2002; Santella et al. 2004). Thus, these three second messengers may contribute to distinct aspects of Ca^{2+} signaling in fertilized eggs of starfish. NAADP may trigger an initial Ca^{2+} increase in the egg cortex whereas InsP_3 contributes to egg-wide propagation of the Ca^{2+} waves (Lim et al. 2001; Santella et al. 2004; Santella and Chun 2013).

13.3.2 *Morphological Changes of the Egg Cortex During Fertilization*

Within a few minutes after fertilization, the actin bundles in the subplasmalemmal zones begin to be translocated centripetally in concert with the internalization of the sperm (Terasaki 1996; Puppo et al. 2008; Vasilev et al. 2012). In addition, the sperm that makes the initial contact with the egg induces the formation of the fertilization cone which is filled with actin filaments (Tilney and Jaffe 1980). Microinjection of starfish eggs with heparin not only inhibits Ca^{2+} signaling but also induces hyperpolymerization of subplasmalemmal actin (Puppo et al. 2008).

These eggs with perturbed actin cytoskeleton exhibit multiple fertilization cones but fail to form the functional tethering that leads to sperm incorporation. Similarly, eggs whose actin meshwork at the plasma membrane is perturbed by ionomycin fail to display the centripetal movement of the F-actin fibers at fertilization and often fail to incorporate sperm (Vasilev et al. 2012).

13.3.3 Changes of the Electrical Property of the Plasma Membrane at Fertilization during Meiotic Maturation

Fertilization also induces rapid changes of the membrane potentials in eggs. After successful binding of sperm, sea urchin eggs either fire a rapid action potential to a positive value of about +20 mV or display a step depolarization (DeFelice and Dale 1979); this is followed by a slower depolarization that initiates simultaneously with the cortical granule exocytosis and elevation of the fertilization envelope. The membrane potential of the fertilized sea urchin eggs remains at positive values for a few minutes but gradually returns to the negative value.

13.4 Block to Polyspermy

Although attracting multiple sperm, the eggs normally incorporate a single sperm *in vitro*. The elevation of the thick fertilization envelope in fertilized eggs of echinoderm led to the idea that such a structure may serve as a mechanical block to polyspermy. However, this process is rather slow; thus, the block to polyspermy may require an additional faster mechanism. A very rapid event that occurs before elevation of the vitelline envelope in fertilized eggs is of special interest. Just (1939) was among the first to describe the possibility of the fast block to polyspermy. He stated, “one can follow the *wave in the ectoplasm* which begins at the point of sperm-entry and sweeps over the egg,” and emphasized that “with membrane-separation, the eggs undergo some change; and it is *this change*—not its result, membrane separation—which constitutes the block to the entrance of additional spermatozoa.” He then added that “this block, which is more subtle than the mechanical obstacle interposed by the presence of a separated membrane, is established *before* membrane-separation occurs.” Indeed, in one of the first experiments to study the nature of the block to polyspermy, Rothschild and Swann (1951, 1952) demonstrated that sea urchin eggs establish monospermic fertilization as early as 2 s after insemination, suggesting that a fast mechanism must be established on the egg surface to reduce the probability of successful refertilization.

As to the physicochemical event underlying the fast block to polyspermy, several hypotheses have been set forth, including those based on the early increase of hydrogen peroxide and protease that would inhibit the binding of supernumerary sperm (Vacquier et al. 1972). A hypothesis that drew special attention proposed that

the fast partial block to polyspermy is electrically mediated by the rapidly changing membrane potential of the egg at fertilization, which renders the egg unreceptive to supernumerary sperm (Jaffe 1976). In support of the view that the establishment of monospermy is linked to the amplitude of the membrane depolarization at fertilization, sea urchin eggs artificially arrested at positive potentials were shown to be prevented from being fertilized by sperm. The “electrical block” hypothesis has been somewhat controversial (Dale and Monroy 1981; Dale and DeFelice 2010). Its main shortcoming, admitted also by its supporters, is that the electrical block is not absolute and can be overridden by high concentration of sperm. Thus, it has been generally agreed that insemination with excess sperm causes polyspermic fertilization in the eggs of many marine animals. However, Just (1939) had already contradicted this idea by showing that eggs of *Arbacia* were never polyspermic even “if a thick sperm-suspension be added to the eggs as early as one second after the first insemination.” He added, “I have also made the initial insemination with the heaviest sperm-concentration procurable, i.e., ‘dry’ sperm as it exudes from the male, and have obtained only mono-spermic fertilization.” He showed that polyspermy in *Arbacia* may occur when the ectoplasm of the fertilizable eggs was injured and thus slowed in reacting to the stimulus of the first sperm. Such injury was very apparent in the eggs with extremely heavy insemination. This observation and the failure of positively held membrane potentials to preclude fertilization of the eggs by excess sperm (Jaffe 1976) imply that an additional factor besides membrane potential may ensure the integrity of eggs and thus guide monospermy. Dale (1985) reported that the sperm receptivity of sea urchin oocytes and eggs was independent of their resting potentials when their jelly layers were kept intact, suggesting that the properties of the egg surface may confer fine regulation of monospermic fertilization.

13.5 Meiotic Stages of Oocytes and Polyspermy

The idea that polyspermy is linked to the regional anatomy of egg surface and cortex can be examined in starfish oocytes at different meiotic maturation stages. As mentioned, the morphology of the starfish oocyte changes with meiotic progression, as judged by the structure of microvilli and cortical actin cytoskeleton. Meiotically immature oocytes are prone to polyspermy upon insemination and fail to elevate the fertilization envelope (Fig. 13.2). They lack an effective fast or slow block to polyspermy (Santella et al. 2012). The progress of cytoplasmic maturation in sea urchin oocytes is also accompanied by structural modifications of the surface layer in a direction that favors monospermic fertilization (Runnström and Monné 1945; Runnström 1963). However, most starfish eggs overmatured beyond the extrusion of second polar bodies display polyspermy at fertilization despite their ability to elevate a normal fertilization envelope (Fujimori and Hirai 1979). The explanation for the polyspermy of these overmature eggs might be twofold. Firstly, the tendency to produce polyspermy may be linked to the electrical properties of the plasma membrane. For instance, the resting potential of the mature eggs of *A. pectinifera* at

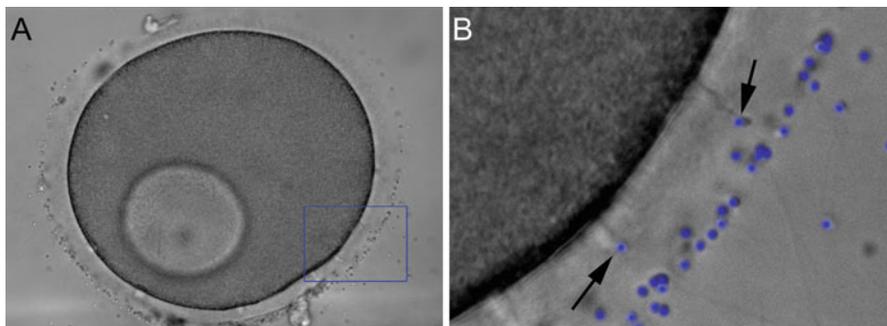


Fig. 13.2 Fertilization of the immature oocytes of *Astropecten aranciacus* at the GV stage. (A) Micrograph of fertilized oocyte 5 min after insemination. Fertilization at this stage leads to formation of multiple fertilization cones, which are indicative of polyspermy. Because of the lack of cortical granules exocytosis, there is no formation of the fertilization envelope. (B) Enlarged view of the region of interest marked with a blue box in A. Fertilizing sperm were stained with Hoechst 33342; sperm making successful contacts are marked with arrows

the optimum period is much less negative (-30 mV) than those of immature (-70 mV) and overmature eggs (-55 mV) because the membrane permeability to K^+ decreases during GVBD but is restored during overmaturation (Miyazaki et al. 1975). The peak of the activation potential in the overmature eggs at fertilization (-4 mV) is also not as high as in the mature eggs at the optimum period ($+12$ mV), rendering them less effective in preventing the entry of supernumerary sperm in the light of the “electrical block” hypothesis (Miyazaki and Hirai 1979; Dale et al. 1979; Moody and Bosma 1985). Second, the increased tendency to polyspermy outside the aforementioned “optimum period” might be caused by the lack of the natural physiological propensity of the egg cytoplasm. Classical studies had indicated that the dispersal of nuclear contents into the cytoplasm and cortex is a prerequisite for cytoplasmic maturation (Delage 1901; Chambers 1921). After the GVDB, a signal or a diffusible substance from the GV may interact with the egg cortex to induce the structural modifications that are essential for egg activation and monospermic sperm incorporation. Indeed, at variance with previous findings (Hirai et al. 1971), the $InsP_3$ -dependent Ca^{2+} response was much slower, with no cortical granules exocytosis in the mature eggs of *A. pectinifera* whose GV had been removed before the addition of 1-MA, whereas Ca^{2+} signaling and elevation of the vitelline layer in response to NAADP and fertilizing sperm were not much affected by enucleation (Lim et al. 2001). On the other hand, eggs matured cortically with low doses of 1-MA displayed an increased rate of polyspermy (Hirohashi et al. 2008). These eggs underwent no GVDB but displayed full elevation of the fertilization envelope. Thus, it is conceivable that either underexposure or overexposure to the nucleus-borne signals during maturation and overmaturation leads to subtle differences in the structure and function of the egg *ectoplasm*, which may disarray the eggs toward polyspermy.

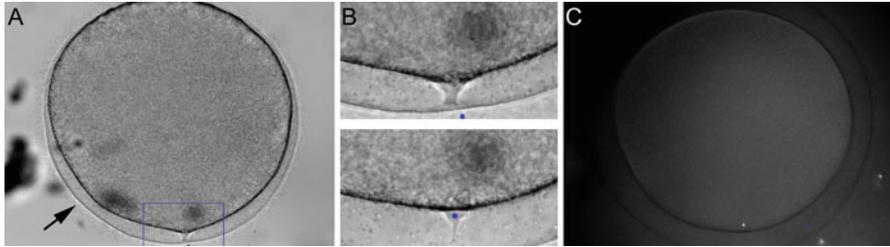


Fig. 13.3 Fertilization of *Astropecten aranciacus* eggs during optimal period. (A) Micrograph of fertilized egg 4 min after insemination. Fertilization at interval between GVDB and extrusion of the first polar body leads to monospermy with formation of one fertilization cone and coordinated elevation of the fertilization. (B) Enlarged views of the sperm entry site. A Hoechst 33342-stained sperm is tethered to the fertilization cone (*top panel*) before being engulfed (*bottom panel*) 3 min later. (C) Visualization of the single Hoechst 33342-stained sperm incorporated into the egg (10 min after insemination)

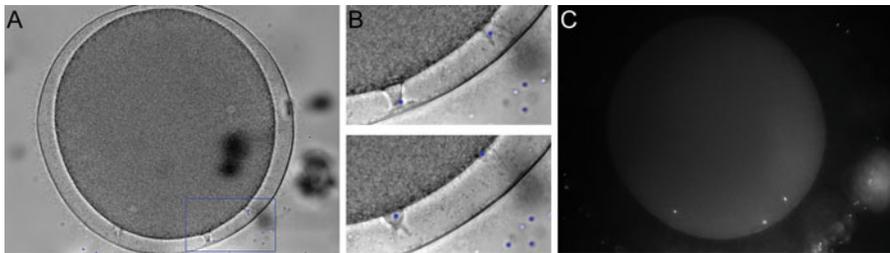


Fig. 13.4 Fertilization of overmature eggs of *Astropecten aranciacus*. Eggs were fertilized 4 h after 1-methyladenine (1-MA) treatment. (A) Micrograph of fertilized egg 4 min after insemination. Although the fertilization envelopes are fully elevated, multiple fertilization cones are formed in most cases. (B) Enlarged view of sperm entry site. Hoechst 33342-stained sperm (*top panel*) are engulfed, as the body of the fertilization cone is detached from the elevating fertilization envelopes 1 min later (*bottom panel*). (C) Visualization of the three Hoechst 33342-stained sperm inside the fertilized egg (10 min after insemination)

The translocation of the cortical granules and vesicles that takes place during the meiotic maturation of oocytes may contribute to shielding the eggs from the attack of supernumerary sperm (Longo et al. 1995). At fertilization, these granules and vesicles undergo exocytosis and their extrusion gives rise to full elevation of fertilization envelope (Fig. 13.3). However, the finding that polyspermy can take place even with full elevation of the fertilization envelope in overmature eggs (Fig. 13.4) indicates that the mechanical block by the fertilization envelope is no guarantee for monospermic fertilization. The fast mechanism preventing the entry of supernumerary sperm may have become disrupted in these eggs. As mentioned, this could be caused by the altered electrical property of the plasma membrane, or may be attributed to the overall failure of the cytoplasmic mechanism in the egg surface that controls sperm interaction and incorporation.

13.6 Role of the Actin Cytoskeleton

One of the most evident morphological consequences of meiotic maturation and overmaturation in starfish eggs is the change of the actin cytoskeleton. Although the signaling pathways leading to it are beyond the scope of this review, the relevance of the actin cytoskeleton to the control of sperm interaction and incorporation may be worth mentioning. Egg microvilli may be involved in deciding sperm receptivity, and their number on the surface of aged mammalian oocytes is severely reduced (Santella et al. 1985). In starfish, a decline in the proper functional interactions between sperm and aged eggs has been suggested (Chambers 1921; Just 1939). Our recent studies on starfish eggs have indicated that the alteration of the structural organization in subplasmalemmal actin filaments renders the egg surface less reactive to the first sperm and more receptive to supernumerary sperm (Puppo et al. 2008; Chun et al. 2010; Vasilev et al. 2012). Eggs with hyperpolymerization of the subplasmalemmal actin displayed the formation of numerous irregularly shaped fertilization cones (thus, polyspermic), yet also displayed unfunctional tethering of the sperm, suggesting a more direct role of actin in sperm interaction and incorporation (Puppo et al. 2008). As mentioned, actin has a modulatory role on the intracellular Ca^{2+} release and influx (Lim et al. 2002; Nusco et al. 2006; Kyojuka et al. 2008; Chun et al. 2010), but it must be emphasized that actin also is a critical factor in controlling exocytosis of the cortical granules. Despite the early claim that intracellular Ca^{2+} elevation is solely important for exocytosis (Steinhardt and Epel 1974; Vacquier 1975; Whitaker and Baker 1983), we have shown that, when the actin cytoskeleton in the cortex is perturbed, intracellular Ca^{2+} increase does not induce discharge of the cortical granules (Kyojuka et al. 2008; Puppo et al. 2008; Chun et al. 2010).

13.7 Concluding Remarks

Although belonging to the same phylum, sea urchin and starfish have evolved to use a fundamentally different temporal scheme at fertilization. Although fertilization of sea urchin awaits the completion of the egg meiosis, natural fertilization of starfish anticipates the meiotic program. To ensure monospermy, the starfish eggs must be fertilized when they are at the incomplete stage of meiosis; that is, during the interval between the GVDB and the extrusion of the first polar body. The reason why starfish evolved to adopt this seemingly premature fertilization scheme has not been clarified, but it may have been inevitable because the same hormone 1-MA not only stimulates oocyte maturation but also promotes spawning of mature gametes (Meijer and Guerrier 1984). Anyhow, the capability of controlling meiotic maturation with 1-MA *in vitro* makes starfish an excellent model system in the study of the relationship among the cellular events that take place during meiotic maturation, fertilization, and egg activation: that is, intracellular Ca^{2+} signaling, dynamic changes of the actin cytoskeleton, and the modulation of ion channel activities.

The rapid rearrangement of the actin cytoskeleton during meiotic maturation and fertilization may be a well-calculated reconfiguration of the cell structure that is linked to the fine regulation of Ca^{2+} signaling, vesicle exocytosis, and sperm incorporation. On the other hand, although the fertilization envelope of the echinoderm egg may provide mechanical protection of the early embryo, the other functions that have been proposed may require careful examination in future studies. The fully elevated fertilization membrane was not sufficient to ensure monospermic fertilization (Fig. 13.4) nor was it a chemical barrier to protect the zygote or early embryo (Vasilev et al. 2012). In view of the relative ease of applying imaging and electrophysiological methods following microinjection, starfish oocytes may provide opportunities to study the reciprocal regulation of cytoskeletal changes and ion channel activities.

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