Chapter 6
Cytoskeletal Elements and the Reproductive Success in Animals

Alessandra Gallo and Elisabetta Tosti

Abbreviations/Acronyms

AR	Acrosome reaction
CG	Cortical granules
GV	Germinal vesicle
GVBD	Germinal vesicle breakdown
MI	Metaphase I
MII	Metaphase II
ZP	Zona pellucida

Introduction

In animals, sexual reproduction is the biological process by which a new individual is generated through the fusion of the gametes, the spermatozoon and oocyte, that are formed during gametogenesis which in turn is underlined by meiosis, the peculiar process of cell division that provides haploid cells ready for fertilization. A correct maturation and reciprocal activation of gametes are pre-requisites for fertilization and, although their temporal and spatial sequences are not yet fully clarified, they involve numerous cellular structures, molecules, ions and metabolic pathways.

In the cell, the shape and structure are due to the cytoskeleton, a complex set of structures composed of microtubules, microfilaments and intermediate filaments

A. Gallo • E. Tosti (✉)
Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy
e-mail: alessandra.gallo@szn.it; tosti@szn.it

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that organize cytoplasmic organelles positioning and intracellular compartments, thus generating cell polarity and contractile forces [1–3].

Microfilaments are composed of actin proteins and play a crucial role in structuring the cell surface and plasma membrane during oocyte maturation and fertilization. They also participate in the maintenance of the meiotic spindle near the cortex, the formation of first and second polar body, the pronuclei apposition and cytokinesis.

Microtubules are tubular polymers of tubulin essential for chromosome movements as well as other aspects of motility and cytoplasmic architecture [4]. In most of the oocytes, microtubules are essential for the first and second meiotic division, furthermore they are fundamental for the swimming of the sperm as well as the union of the male and female pronuclei.

Many studies have been devoted to examine the factors that may influence the success of fertilization. This chapter will discuss the modification of gamete ultrastructure during the processes of oocyte and sperm maturation and fertilization, focusing on the crucial role of the cytoskeletal structures in ensuring a successful fertilization and normal embryo formation in some key species of marine invertebrates and mammals.

The Gametes

Oogenesis and spermatogenesis are characterized by meiosis, the unique process of cell division occurring only in gametes, whose goal is the production of haploid cells highly specialized for fertilization.

The Spermatozoon: Structure and Maturation

The primary function of the spermatozoon is to deliver the male genetic material into the oocyte to generate a new diploid individual. The success of fertilization depends on a series of processes based on a correct maturation of the spermatozoon, its transport toward a receptive oocyte and the ability to recognize and fuse with it. In order to perform these functions, the spermatozoon has developed a highly specialized morphology with different structural components each aimed to a specific processes. Basic structure of the spermatozoon is common to almost all the species and includes three major parts [5]: (1) the head, that is the site for recognition and fusion, has a roundish shape and contains a few structures such as the nucleus, scant cytoplasm and the acrosome that is a cap-like structure over the anterior half of the head; (2) the midpiece is located at the base of the head and includes the centrioles, few mitochondria, axoneme base and related anchoring structures; and (3) the tail is a long flagellum composed of an axoneme, a highly organized microtubule-based structure composed of about 250 proteins. A typical structure (9+2) is made up of

peripheral doublets of longitudinal microtubules on which are fixed dynein arms and radial spokes and a pair of singlet microtubules located in the central core.

Unlike the oocyte, the spermatozoon undergoes the final step of the maturation process (spermiogenesis) through a dramatic change in shape and morphology passing from a round cell to the characteristic tadpole aspect.

An important feature of spermatogenesis is the change in the cytoskeleton that occurs throughout this pathway. Although primary focus is given to the microtubule cytoskeleton, the importance of actin filaments to the cellular transformation of the male germ cell has also been shown [6].

During spermatogenesis, diverse processes occur such as sequential changes in the nucleus and the acrosome in concert with a prominent bundles of microtubules called the manchette, a high condensation of chromatin in the nucleus and a cytoplasmic remodelling of the sperm body and structures [7, 8].

Actin has been shown to cooperate in various aspects of the spermatogenesis along with myosin, an actin-dependent motor protein. Actin is present in the form of monomer, oligomer and polymer within cells, the latter are called microfilaments and are involved in the shaping and differentiation of spermatids.

Three major cytoskeletal proteins, actin, actin-binding proteins such as spectrin and various tubulins (e.g. a-, b-, g-tubulin), are present in the head of mammalian spermatozoa with a pattern similar for all the species. Changes in localization of cytoskeleton support the image of cytoskeletal proteins as highly dynamic structures participating actively in processes prior to fertilization [9, 10].

During spermatogenesis, the actin cytoskeleton shows active remodeling. Some actin binding or actin regulated proteins have been demonstrated to regulate dynamic changes of the actin-containing structures. Myosin plays also an important role in acrosome biogenesis, vesicle transport, gene transcription and nuclear shaping [11].

The Oocyte: Structure and Maturation

The oocyte is the large cell characterized by a single function of generating a new individual. The general organization of a mature oocyte is similar along the species but sometime shows unique features [12], in fact, the oocyte is surrounded by extracellular membranes that appear to be thin in the sea urchin (the vitelline layer) [13] or tick in ascidians (the chorion) [14] and mammals (the zona pellucida; ZP) [15].

The oocyte plasma membrane marks the borderline between the internal and external compartments, represents a barrier to ions whose passage occurs towards the ion channels, specific proteins located inside the lipid bilayer [16]. Plasma membrane has many extensions, called microvilli, involved in the fusion process [17]. The cortical granules (CG) are round organelles situated in mono and multiple layers under the cell plasma membrane. CG originate from the Golgi complex and contain mainly enzymes and mucopolysaccharides; although they show a high variability in shape and size, they are present in the oocytes of most of the animals,

playing a role during oocyte activation when they release their content by exocytosis [18]. The mitochondria are organelles playing key roles in the oxidative cellular energy metabolism. Their number and ultrastructural organization change with cell function and activity, infact oocytes, zygotes, and embryos present particular types of mitochondria [19].

Oocyte maturation is a complex process occurring at the end of oogenesis during which the oocyte completes its growth and undergoes a series of changes that are necessary for ovulation, fertilization and early embryo development. In the stage that precedes maturation, the oocyte is a large round cell with a big nucleus called germinal vesicle (GV) that at very early stage contains decondensed transcriptionally active chromatin [20].

Oocyte maturation involves two different but interlinked processes based on nuclear and cytoplasmic events [21–23].

Nuclear maturation starts when the oocyte under a chemical stimulation resumes meiosis inducing the breakdown of the GV (GVBD), the chromosome condensation and the spindle formation. A second meiotic arrest then occurs at different stages depending on the species such as metaphase I (MI) in ascidians, bivalves and gastropods, whereas mammalian oocytes complete the first round of meiosis with extrusion of the first polar body and, without an interphase, progress to the second meiotic metaphase (MII). Apart from some exceptions, this process is completed upon fertilization in almost all the species studied.

Cytoplasmic maturation is a less clear process that starts at the time of oocyte growth and occurs with the following events: (1) ultrastructural organelle re-organization; (2) molecular modifications of the plasma membrane; (3) differentiation of the calcium signalling machinery and (4) oocyte surface microvilli increase. Ultrastructural reorganization involves the redistribution of microfilaments and microtubules that in turn support the relocation of cytoplasmic organelles such as cortical granules, mitochondria and the Golgi apparatus in several species [18, 20, 24–35]. In particular mitochondria redistribution appears to be functional to oocyte developmental competence and the regulation of normal embryo development [36].

Fertilization

Fertilization is a highly specialized process of cell to cell interaction that marks the creation of a new and unique individual [23, 37]. The main steps of the fertilization process are gametogenesis, gamete reciprocal activation, sperm-oocyte interaction, fusion and syngamy, thereafter these successful events give rise to the beginning of development.

Reciprocal activation of gametes is fundamental for a successfully fertilization. First the oocyte induces sperm activation due mainly to the signals coming from the oocyte investments [38]. After a series of processes, the activated spermatozoon reaches the oocyte exerting its dual function: to transport the male genome into the oocyte and to trigger the quiescent oocyte into activation [39]. The latter is

underlined by another series of events, that include electrical, structural and molecular changes, up to the release of oocyte from meiotic arrest [40] giving rise to the zygote, the first cell of the living organism that becomes an embryo after a series of mitotic divisions.

Cytoskeletal Elements Modulating Gamete Activation

The Spermatozoon

Sperm activation triggers in progression: (1) the sperm motility (chemokinesis); (2) the attraction toward the oocyte itself (chemotaxis); (3) the first binding mediated by ligands and receptors on the gamete plasma membrane; (4) the acrosome reaction (AR); (5) the penetration through the extracellular layers; (6) a second binding; and (7) the fusion of the two plasma membranes.

Sperm motility is required for sperm transport toward the oocyte, either in the aquatic environment or the female genital tract. Sperm motility is an essential condition for male fertility and is fully underlined by the tail. A flagellar movement is provided by the sliding of adjacent microtubules thanks to the ATP hydrolysis occurring in the mitochondria located on the close midpiece. In particular, the propagation of a wave is repeated along the flagellum by a mechanochemical cycle of attachment-detachment of dynein arms giving rise to the flagellar sliding and bending [41–43].

Recent proteomic analyses have provided insight into novel cellular and functional aspects of sperm actin isoforms in the axoneme of ascidians [44].

Motility initiation and hyperactivation are also supported by other specific cytoskeletal elements and dynamics such as major sperm protein (MSP) filaments in the nematodes [45, 46] and polymerization of actin in mammals [47, 48].

Chemotaxis is the process by which spermatozoa are attracted by microenvironmental factors mainly released by the oocyte or perioocyte layers [49]. To provide a more efficient motility, chemotaxis generates dramatic movement changes induced by the interaction of external factor with membrane “receptors” and intracellular messengers such as cyclic AMP, ATP, calcium, or pH changes. All the signaling molecules involved in this process are closely arranged in the sperm flagellum controlling dynein-microtubules interaction through a phosphorylation-dephosphorylation process of axonemal proteins [50].

The first contact between the two gametes is the binding of the sperm to the extracellular investments of the oocyte, this is a receptor-ligand interaction with a high degree of species- specificity that allows to prevent fusion of sperm and oocytes of different species. The carbohydrate groups on the oocyte surface function as sperm receptors. The sperm molecules that bind this receptor are not known with certainty, and indeed, there may be several proteins that can serve this function. In mammals the first association of the spermatozoon with the ZP occurs between the zona glycoprotein, ZP3, and sperm receptor, located on the sperm plasma membrane,

such as the 95 kDa tyrosine kinase-protein. This interaction induces the AR [51]. The latter is an exocytotic process mediated by calcium occurring at the fusion of the outer acrosomal membrane with the sperm plasma membrane. The breakdown of the fused complex results then in the formation of a highly fusible membrane enabling the spermatozoon to penetrate into the oocyte and fertilize it.

Numerous cytoskeletal elements and proteins appear to be involved in either binding and AR, involving mainly the actin and mediated by numerous second messengers. Data suggest that actin polymerization may represent an important regulatory pathway which is associated with tyrosine phosphorylation in spermatozoa [52].

In echinoderms, the most important event which occurs during the AR is the polymerization of actin, which form the "skeleton" of the acrosomal process, a protuberance formed at the apex of the sperm head supported by a core of actin microfilaments [53, 54]. Apart this peculiar event in echinoderms, many authors have suggested that the acrosomal architecture is supported by a dynamic F-actin skeleton, which probably regulates the differential rate of release of the acrosomal enzymes during AR [55].

Changes and regulation of the sperm actin cytoskeleton in fact, take place during AR; in mammals, polymerization of actin from its globular (G)- monomeric form to filamentous (F)-actin occurs during capacitation, depending on phosphorylation processes. F-actin formation is important for the translocation of phospholipase C from the cytosol to the sperm plasma membrane during capacitation. Before the occurrence of AR, depolymerization of F-actin enables the outer acrosomal membrane to fuse with the plasma membrane [52, 56]. In support of this finding, an important role of actin polymerization has also been shown in human sperm AR since actin is present in the acrosomal area and is lost with the AR [57]. In human and other mammalian spermatozoa, cytoskeletal proteins including spectrin, F-actin and α-tubulin were mostly localized to the apical and the equatorial acrosomal region of the sperm head, and their modification after AR was evidenced suggesting, at least, that they may play more than a role in the development of the AR and priming the spermatozoa for other fertilization events [9, 10].

After AR is completed, the spermatozoon can begin penetration through the extracellular layers. Penetration may involve enzymatic hydrolysis of the extracellular matrix but also requires the forward physical force of sperm motility [58].

More specific structure are present on the sperm head of murids where two very large cytoskeletal structures seem to be involved in binding of the spermatozoon to the outer surface of the ZP and/or in aiding the spermatozoon in ZP penetration at the time of fertilization [59].

The possible role of actin filaments in the penetration of spermatozoa has been evidenced by indirect proofs in mammals since cytochalasin D inhibits sperm penetration and sperm head decondensation [60]. Similar investigations in mammals proved that either actin polymerization [61] and Rho protein(s) regulating actin-based cytoskeletal reorganization are involved in the process leading to sperm incorporation into the oocyte cytoplasm [62].

Once the spermatozoon penetrates the oocyte a fusion of the two plasma membrane occurs.

Sperm penetration occurs vertical to the surface of invertebrate oocytes possessing a jelly coat, whereas in mammals, sperm lies and fuse tangential to the oocyte surface [63]. In the latter, although the exact sperm fusogenic region is not fully established, studies suggest that this is a region overlapping either the equatorial segment or the postacrosomal region depending on the species under study [23]. Despite the importance of this fundamental process, little is known about its molecular basis. Although a number of molecules involved in the binding and fusion have been disclosed [64], indirect evidences supported the Izumo, a sperm-specific member of the immunoglobulin superfamily which relocalization to the equatorial segment after the AR is essential for gamete fusion and the testis-specific serine kinase 6 that plays a role in the changes of Izumo localization through the regulation of actin polymerization [65, 66]. Contrasting data from other authors [61] showed that, although involved in sperm penetration, actin polymerization is not required for plasma membrane gametes fusion in guinea pig.

Very recently, experiments aimed to investigate a role of proteins enriched in the cytoskeletal structures of human spermatozoa demonstrated that signal transducer and activator of transcription 3 (STAT3), present mainly in the flagellar structure, affects sperm functions such as motility parameters, AR and depolarization of mitochondrial membranes [67].

Evidences were presented of an involvement of organellar movements in the ascidian spermatozoon. This lacks an evident acrosome and midpiece but presents a single mitochondrion beside the nucleus in the head that swells at the time of oocyte interaction being translocated to the tail. Such a movement appear to be mediated by an actin-myosin sliding system [68]. To conclude, a very recent computational and experimental approach pointed out that the "actin polymerization" have some important and unique features by linking in a specific way all the intracellular compartments. Thus, it was suggested that actin polymerization could be involved in the signaling coordination of different events and that its functional ablation could compromise spermatozoa ability to complete the capacitation. This study strengthen the idea that the actin cytoskeleton is not only a mechanical support for the sperm cell, but that it exerts a key role in signaling during capacitation [69] (Fig. 6.1).

The Oocyte

In oocyte maturation, GVBD represents the nuclear event strictly related to the first meiotic block resumption and the following meiotic spindle formation. The involvement of cytoskeletal elements to GVBD has been investigated in several species.

In some mammals, actin filaments are distributed in a uniform way just around the oocyte cortex and close to the GV, and undergo a redistribution after the GVBD leading the chromosome to move to a peripheral position [70–74]; however this event does not seem to influence either GVBD or the spindle formation [32]. The presence of a cortical "organizing pole" of microfilaments has been hypothesized in the maturing mouse oocytes especially during centrosome localization,

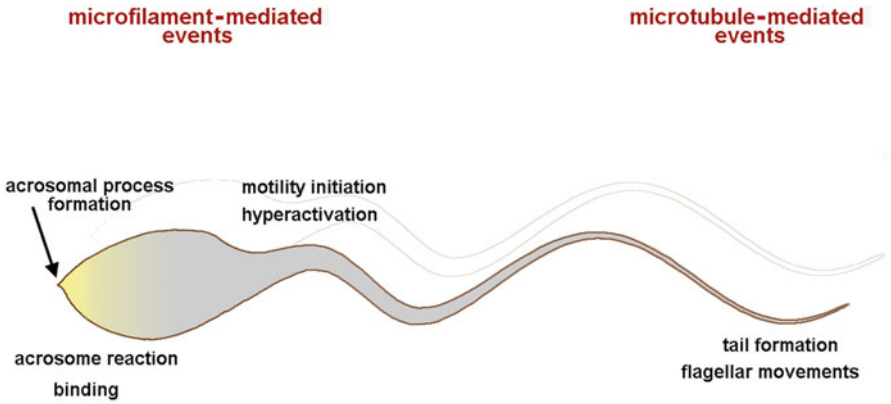

Fig. 6.1 Involvement of cytoskeletal elements during spermatozoon maturation and activation. Microfilaments are involved in the acrosome reaction, formation of the acrosomal process, first binding to the oocyte, motility initiation and hyperactivation. Microtubules are the constituent of sperm flagellum that is formed during the process of spermiogenesis and allow the flagellar movement

spindle (or GV) movement to the oocyte periphery [75]. In other vertebrates, such as amphibians, contrasting data exist on the role of cortical actin microfilaments that appear to be required for anchoring and rotation of the meiotic spindles [76] and the completion of GVBD [77]. In the ascidian *Halocynthia roretzi*, it has been identified the formation of conspicuous actin bundles emanating from the GV during its breakdown [78], and same authors showed that after GVBD a meiotic spindle forms in the center of the oocyte migrating toward the animal pole requiring actin cytoskeleton to support the polarization [79]. Although the latter event occurs normally in the absence of microtubules, cytoskeletal elements interact by each other giving rise to a fruitful interplay; this is the case of actin filament modulation of microtubules functions that drive chromosomes segregation in the mitotic and meiotic spindles, their positioning and orientation, processes that appear to be essential for the asymmetric cell division [80–83]. In fact, the synergy between microfilaments and microtubules has been supported by experiments based on specific chemicals such as cytochalasins [74, 84] in mammals. Similarly in amphibians, data are provided on the involvement of actin filaments in spindle anchorage [76, 85] and of myosin-10 (a phosphoinositide-binding actin-based protein) in association with microtubules in vitro and in vivo, with a specific localization at the point where the meiotic spindle contacts the F-actin-rich cortex [86].

The meiotic spindle consists of bundles of microtubules that emanate from two acentriolar poles and hold chromosomes along the metaphase plate. At meiosis resumption the spindle segregates sister chromatids or homologous chromosomes equally between the pronucleus and the second polar body playing a critical role in the generation of right chromosome segregation [87, 88]. Literature reports the requirement of microtubule associated motor proteins, for the proper distribution of chromosomes or the structural integrity of the mitotic or meiotic spindle [89].

Interestingly, it has been shown that a microtubules perturbation induces negative impact on GVBD and the meiotic resumption [90].

The interaction of the spermatozoon with the oocyte causes a series of physiological changes in the oocyte known as activation. An early event that occurs at fertilization is the change in the oocyte plasma membrane electrical properties [16, 39, 91] and the second main universal event is the massive release of calcium that traverses the oocyte in a wave [92–94], leading also to relocation of the organelles.

Organelles that have been organized in specific sites of the oocyte during growth and maturation undergo a relocation at the oocyte activation. In the ascidian *Styela plicata* we reported a pattern of mitochondria polarization and aggregation in the subcortical cytoplasm during oocyte growth [95]. These data strongly support what occurs in ascidian oocytes at the time of activation when the subcortical mitochondria are transported to the vegetal pole [96, 97]. This process called cytoplasmic segregation, that is necessary for the establishment of cell lines and in turn for determining the embryonic axis, involves myoplasmic actin-filaments network in a first phase whereas in the second phase involves extension of microtubules [14].

On the other hand the requirement of actin in the first phase after sperm contact has been shown [98] since perturbation of fertilization in ascidians with specific channel inhibitors altered either actin filaments and mitochondrial migration after contraction leading to a disturbance in the following cleavage formation. The regulation of mitochondrial translocation by microfilaments and microtubules observed in mammals indicated that either oocyte maturation, fertilization and early embryo development in pigs are associated with changes in active mitochondrial distribution and that this is mediated exclusively by microtubules [99]. However more recent evidence also indicates that the cytoskeleton network is used to shuttle organelles to specific sites within the oocyte cytoplasm [100].

Following the fusion of the spermatozoon with the oocyte plasma membrane, a third event occurs when the oocyte secretes the contents of CG by exocytotic fusions of these vesicles with the oocyte plasma membrane over the entire cell surface, also known as the cortical reaction or CG exocytosis [101]. This peculiar process is followed by an elevation or hardening of the extracellular coat involved in the polispermy prevention in sea urchin and mammals, however it does not occur in ascidians since their oocytes lack CG. Many cell types possess finger-like projections termed microvilli. In the sea urchin an ultrastructural study localized filamentous actin immediately subjacent to the microvilli forming an extensive interconnecting network along the inner surface of the plasma membrane with an organization of this network correlated to the positioning of the underlying CG [102]. That the microfilament assembly is involved in the distribution, movement and exocytosis of CG during maturation and fertilization has been shown by confocal microscopy in the pig oocyte. Here, it was suggested an integral changes in microfilament assembly and CG distribution during oocyte maturation, parthenogenic activation and in vitro fertilization [103]. Similarly in the rat it was supported the role of cytoskeletal cortex as a dynamic network that modulate CG exocytosis by activated actin-associated proteins and/or by activated protein kinase C [104].

As a consequence of cortical reaction the thousands of vesicles fusing with the oocyte surface, add their membranes to the oocyte plasma membrane resulting in an approximate doubling of the amount of membrane on the oocyte surface in a few seconds with the production of a mosaic topography, so the excess surface membrane is therefore accommodated by the elongation of oocyte microvilli [105, 106].

Earlier studies in the 1980 showed a dramatic reorganization occurring in the structure of the oocyte surface due to the mosaic membrane formed after activation and the resulting elongation of numerous short microvilli that covers the surface of the unfertilized oocyte. A localization of actin in the microvilli has been also deeply investigated in sea urchin species showing the formation of bundles of actin filaments in microvilli and in cones [107]. This suggested that this microvillar-associated actin was an organizational state composed of very short filaments arranged in a tight network and that these filament networks were extended beyond the plane of the plasma membrane [108].

In the cortical region of amphibian and rat oocytes it has been shown a significant amount of polymerized actin organized into bundles within the short microvilli covering the oocyte surface [109]. In the sea urchin, morphological studies evidenced two bursts of microvilli elongation concomitantly to sperm entering and incorporation, as a result of a massive polymerization of actin and a new assembly of microfilaments in the oocyte cortex reorganization that was suggested to produce the forces necessary to held firmly the spermatozoon for fusion and subsequently for cytokinesis occurrence [54, 110, 111]. More recent studies in mammals, further support the role of cytoskeletal actin in microvilli formation and their function to capture the sperm cell and bring it into close contact with the oocyte plasma membrane [17]. This data should also support the fact that in some mammalian oocytes the spermatozoon do not normally fuse with the microvillus-free area [112].

That sperm incorporation is a microfilament-dependent process has been shown in Xenopus [113] but this process has been described to also occur through the formation on the oocyte surface of a specific structure named the fertilization cone involving the functioning of actin microfilament organization. This process is related to the overmentioned elongation of microvilli and has been described to occur in the echinoderms [114–117]. In the sheep at the site of sperm head incorporation, the fertilization cone develops above the decondensing male chromatin and is underlined by a submembranous area rich in microfilaments [118].

Once the sperm has entered into the oocyte, the proximal centrosome adjacent to the sperm nucleus may become the center of the sperm aster that brings the male and female pronuclei to the center of the zygote [119]. Aster is a peculiar structure that appears initially after the centriole duplication at the pronuclear stage required for the union of the sperm and oocyte nuclei and is formed by the assembly of the microtubules mainly composed by the g-tubulin which is also needed for the subsequent enlargement and association with the female pronucleus [22, 120, 121]. In the rabbit, earlier studies showed the presence and continuous deposits of tubulin throughout the sperm penetration tunnels and entry point suggesting a role in fertilization, possibly as an enzyme binding or delivery system [122]. More recently it was shown that the microtubules extending from the decondensed sperm head

participating in pronuclear migration and organization around the female pronucleus resulted mainly composed by γ-tubulin [123].

Among the events occurring at oocyte activation the change in the ionic permeability of the oocyte due to the generation of a ion current across the plasma membrane and calcium release play a pivotal role. Depending on the species, specific and nonspecific ion currents are involved at the early sperm-oocyte interaction [16, 124]. It is well established that actin filaments are important in ion channel regulation and membrane potential modulation [125, 126], although this does not seem to be the case of ascidian oocytes since actin filaments have no impact on the fertilization current or plasma membrane [98]. Indirect evidences support that spectrin, a major component of the membrane skeleton, is a functional link with membrane channels and transporters [127, 128].

Calcium release is the universal event occurring at fertilization in all species studied [94]. In the ascidians, fertilization at MI initiates a series of dramatic cytoplasmic and cortical reorganizations of the zygote, which occur in two major phases [129]. The first major phase depends on sperm entry which triggers a calcium wave leading in turn to an actomyosin-driven contraction wave. The second major phase of reorganization occurs between meiosis completion and the first cleavage. Sperm aster microtubules and then cortical microfilaments cause the reposition toward the posterior side of the zygote of myoplasm and of domain rich in cortical endoplasmic reticulum and maternal RNAs [130].

The possibility that intracellular calcium signaling could be modulated by the actin cytoskeleton at the time of gamete interaction has been also recently hypothesized in starfish [131, 132] whereas in the sea urchin the calcium-responsive contractility during fertilization is modulated by the myosin II localized to the cortical cytoskeleton. This seems also to influence the fertilization cone absorption and to participate in the remodeling of the cortical actomyosin cytoskeleton during the following first zygotic cell cycle [133]. Finally a coordinated mobilization of intracellular calcium stores and a precise organization of the cytoskeletal network have been shown to be essential for an appropriate activation of the oocyte and chromosome migration during human fertilization [134].

Post-fertilization events include the sperm cell nucleus breakdown and chromatin decondensation that is then surrounded by an envelope forming the male pronucleus. The latter, together with the female pronucleus located just below the extruded polar body, start to move toward the center of the oocyte. These processes are under the influence of factors in the cytoplasm. Emission of the polar body due to meiosis resumption has been shown to be underlined by the formation of a contractile ring of actin in the cleavage furrow of the asymmetric division of the oocyte [70, 135] whereas migration of the pronuclei depends strictly on the microtubules of the sperm aster [63]. Rotation of meiotic spindle is under the control of microfilaments [76, 136] but a peculiar interplay between astral microtubules and cortical actin filaments has been suggested for spindle positioning [137–139] and pronuclear apposition. Although a main role of F-actin in the formation of contractile ring during the first cleavage division has been well documented [140], the cooperation between the two main cytoskeletal elements has also been identified in the Xenopus [141] (Fig. 6.2).

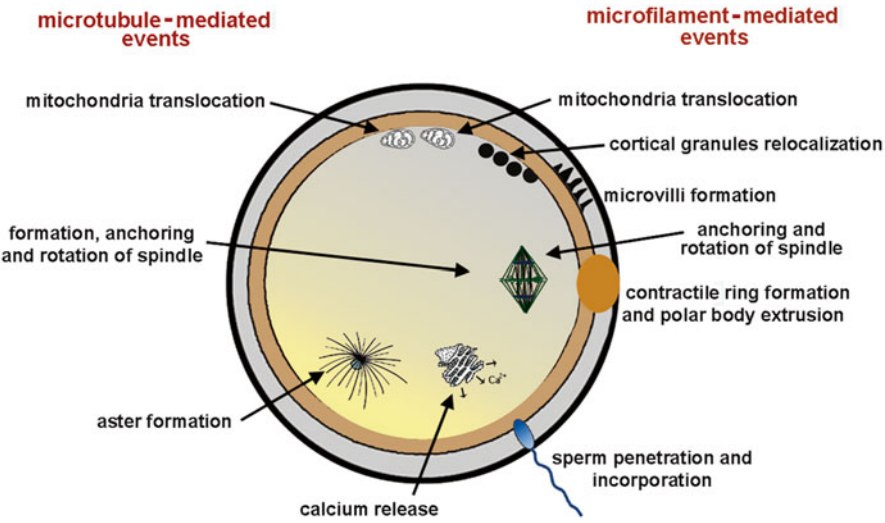

Fig. 6.2 Involvement of cytoskeletal elements during oocyte maturation and activation. Microfilaments are involved in the cortical granules relocalization during growth and the formation of microvilli just after the exocytosis of cortical granules occurring at fertilization. They also participate to formation of contractile ring that in turn give rise to the polar body extrusion. Sperm incorporation and penetration in the oocyte and the following calcium release are also modulated by actin microfilaments. Microtubules are involved in the formation of meiotic spindle and of sperm aster that drives the male pronucleus toward the female one after fertilization. Either microfilaments and microtubules participate to the mitochondria translocation during maturation and to the anchoring and rotation of the meiotic spindle after the sperm-oocyte interaction

The Zygote

Right embryo development relies on the positioning of the cleavage plane which is in turn related to the position of the mitotic spindle. In the mouse zygote it has been demonstrated an accumulation of F-actin surrounding the spindle and that actin network maintains the central spindle position ensuring that the first embryonic mitosis is symmetric [142]. However, in the establishment of the right symmetry in cell divisions during differentiation and subsequent embryo development a central role is also played by the paternal centrosome [143] whose role and involvement of cytoskeletal elements has been previously reported. In fact, in bovine evidence have been provided that γ-tubulin and microtubule dynamics are involved in the migration and centration of the female pronucleus [144]. On the other hand indirect evidences exist that perturbation of tubulin polymerization induces meiotic delay and spindle defects contributing to formation of aneuploid mouse zygotes [145]. By contrast in human zygotes showing abnormal fertilization, no any kind of microtubule alteration with respect to the ploidy level was observed [146]. Also in the invertebrates the cortical actin cytoskeleton undergoes dramatic rearrangements with a level of F-actin decreasing after fertilization and continuing to decrease

throughout the first cell cycle of sea urchin [147]. Such a dynamic nature of cortical actin organization during early development demonstrated also that cytokinesis occurs at the point of minimum cortical F-actin content suggesting that these changes do not function in the establishment of the contractile apparatus for cytokinesis, but rather serve other developmental functions [148]. Similarly in ascidians the determinants for unequal cleavage, gastrulation and further developmental events reside in four distinct cortical and cytoplasmic domains localized in the oocyte between fertilization and first divisions [96].

Conclusions

Involvements of cytoskeleton in reproductive processes have received the special attention of many authors. The related large body of literature shows an impressive variation along the species, however, common general characteristics of the process emerge, allowing to depict a general picture of the complex interplay between cytoskeletal elements and the physiology of fertilization. The cytoskeleton has a fundamental role in numerous cellular processes, consequently, it has been shown that abnormalities in the regulation of cytoskeleton dynamics are typical for many pathological states from infection processes up to cancer [149–151]. Fertilization is a multistep process in which all physiologically relevant events are intimately connected with each other and in turn are crucial for the entire process of reproduction; therefore it appears that only the right combination of multifactorial causes brings to a normal embryo and organism development. Here, we have reported that cytoskeletal elements as microfilaments and microtubules are involved in all the steps from the maturation of gametes, their reciprocal activation to the final interaction and the initiation of embryo development. Although sometime results are controversial and come from indirect experimental data, evidence are provided that perturbation of the cytoskeleton, with toxins or heath shock, exerts a wide range of impacts on the entire reproductive process including sperm maturation and motility, oocyte maturation, fertilization and embryo development [152–154]. Studies on human in vitro fertilization evidenced the delicate nature of the oocyte and the instrumental role played in fertilization reinforcing the view that: (1) exposure to mechanical stressors has the potential to compromise oocyte developmental competence; (2) defects in any of the aforementioned reproductive events are lethal to the embryo development and might be causes of infertility; (3) cytoskeletal dynamics perturbation of gametes may be considered a factor of human infertility [124, 155–161]. In this chapter, we wished to bring the general concepts that the major cytoskeletal structures are involved in the reproductive processes. We would like to apologize with the colleagues for not having reported all their valuable studies on animal models such as drosophila, zebrafish, nematodes etc., but given the vastness of the literature on a variety of animal species we have chosen to deal about those species which have always been models for the study of reproduction including either invertebrates and vertebrates.

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