Ion currents involved in gamete physiology

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ABSTRACT Gametes are electrogenic cells that modify their electrical properties in response to different stimuli. This behavior is due to the occurrence of ion currents flowing through ion channels located on the plasma membranes. The modulation of ion channels has been described during the processes of gamete maturation, activation and fertilization in most of the animal models studied. In particular, predominant ions involved in physiological events in oocyte and sperm have been recognized to be sodium, potassium and calcium. In this review, we give an overview on the occurrence, modulation and function of ion fluxes, from gametogenesis to early fertilization events, from marine animals to human. The implications for a dynamic role of ion currents in gamete physiology and their possible clinical and technological applications are discussed.

KEY WORDS: *gamete, ion channel, ion current, oocyte, spermatozoa*

Introduction

Main steps of the reproductive process are the production of the two gametes (spermatogenesis and oogenesis), their reciprocal activation and the following interaction (e.g. fertilization). Gametogenesis is underlined by meiosis, the unique process of cell division which provides the formation of haploid spermatozoa and oocytes. Gamete maturation is the last phase of gametogenesis and transforms immature cells in gametes competent for fertilization. This is the highly specialized process of cell interaction and signal transduction that generates a new individual of the same species through different events, such as recognition, binding and fusion of the two gametes. These events are also accompanied by a still unclear process that is the reciprocal activation of the gametes. First the spermatozoon is activated by the signals coming from the oocyte extracellular membranes, that induce changes either in the shape and function of the spermatozoon rendering it a motile and fusible cell and attracting it toward the oocyte. At the end of the sperm activation and just after the fusion of the two plasma membranes, the spermatozoon starts to activate the oocyte by inducing calcium (Ca^{2+}) oscillations (Gillot *et al.,* 1990; Swann and Yu 2008) transforming its quiescence into a metabolic activated state up to cell cycle resumption (Dupont *et al.,* 2010) that in turn triggers the program of development (Yanagimachi 1994).

Gametes are excitable cells which, during the processes of maturation and fertilization, undergo transient modifications of the electrical properties due to the activity of ion channels present on their plasma membrane. The modulation of ion channels have been widely demonstrated on the oocytes and spermatozoa of all the animals studied (Darszon *et al.,* 2001; Hagiwara and Jaffe 1979; Tosti and Boni 2004; Tosti *et al.,* 2013). The modifications in the plasma membrane asset have been historically first studied in marine invertebrates and then extended to mammals and human.

In this review, we will describe the presence and activity of currents flowing through the ion channels in the oocyte and spermatozoon and in the early stages which follow their interaction in the most studied animal species from marine invertebrates to human (Figs. 1,2).

Why study the plasma membrane?

The plasma membrane marks the borderline between inner and outer compartments of the cell. On the gamete plasma membrane there are located all the molecules (ligand and receptors) responsible for the events of recognition, binding and fusion. Ion channels are among these molecules and their involvement in the functionality of gametes is a matter of intense study. The plasma membrane is the site of a difference of electrical charge distribution that creates an electrical gradient named voltage. The voltage difference across the plasma membrane creates a store of potential energy that give rise to a trans-membrane potential known as resting potential.

Every excitable cell has a specific resting potential that is normally negative ranging from -10 millivolt (mV) to -100 mV (DeFelice

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1997). The voltage is due to a diverse distribution of ions inside and outside the cell. Among main important ions, potassium (K^+) is more concentrated inside the cell, whereas Ca^{2+} and sodium (Na^+) are in the extracellular environment.

The bilayer is impenetrable to the free diffusion of the ions that may cross it only through the ion channels. These are pore-forming proteins with complex structures characterized by the properties summarized below:

i) specificity: is the passage of only one specific ion; ii) gating: is the modulation by either change of voltage or a ligand/messenger; iii) conductance: is the amount of ions that may pass thorough them; iv) sensitivity: describes what molecules/drugs are able to modulate their gating.

With the advent of the patch clamp technique in 1976 (Neher and Sakmann 1992), it has been possible to record the ion currents flowing through the cell, depending on the characteristics of the ions and the gradient across the membrane. Whole-cell and single channel recordings of channel activity are the basic configurations of the patch clamp technique. They are obtained after a seal of the plasma membrane with the glass electrode connected to the electrophysiological apparatus, but only the whole-cell configuration requires the destruction of the patched membrane to obtain free access to the cytoplasm. The single channel configuration allows a recording of the channel activity inside the intact patched membrane. The study of ion currents has provided basic information about cell physiology and function.

Ion currents in gametes

The oocyte

Oocytes are quiescent cells arrested at different stages of the meiotic cycle depending on the species. Earlier studies on the role of ions in the oocyte were performed on the sea urchin by Edward Chambers (Chambers 1989) who investigated the ionic exchange of Na^+ and K^+ at fertilization. Among echinoderms, sea urchin and starfish have been extensively studied demonstrating a wide variety of ion current activities in their oocytes.

After the pioneer studies of Chambers, mature sea urchin oocytes were investigated by several authors who better characterized the ion activity showing a high permeability to K^+ and a lower permeability to Na^+ (Jaffe and Robinson 1978; Steinhardt *et al.,* 1971). Following studies (Dale and de Santis 1981) demonstrated a decrease of K^+ permeability along oocyte maturation accompanied by a decrease in the membrane conductance. The presence of Ca^{2+} current activities was instead firstly demonstrated in a comparative study (Okamoto *et al.,* 1977).

Immature oocytes of different starfish species were studied in the early '80 especially pointing on the modification of the electrical asset associated to the 1-methyladenine induced maturation (Moreau *et al.,* 1985).

Oocytes of *Leptasterias hexactis* exhibited three types of voltage-dependent currents, i.e. an inward Ca^{2+}, a fast transient K^+ and an inwardly rectifying K^+ current, that gradually changed during *in vitro* maturation, in particular the Ca^{2+} current become larger whereas both K^+ currents become smaller (Moody and Bosma 1985; Moody and Lansman 1983). The changes in the ion current kinetic were particularly evident at the end of the growth phase and were associated with the migration of the germinal vesicle (GV) at the periphery of the cell (Moody 1985).

Invertebrates	Gamete	Currents/Channels	Refs.
Echinoderms Sea urchin		K^+, Na^+ Ca^{2+}	Dale & De Santis, 1981 Jaffe & Robinson, 1978 Okamoto et al., 1977 Steinhardt et al., 1971
		K^+, Na^+ Ca^{2+}, Cl^-	Chan et al., 1997 Kirichok & Lishko, 2006 Lishko et al., 2013 Reyes et al., 1994
Starfish		K^+, Na^+ Ca^{2+}, Cl^-	Dale et al., 1978 Miyazaki et al., 1975 Moody & Bosma, 1985 Moody & Lansman, 1983 Moreau & Cheval, 1976 Shen & Steinhardt, 1976
Ascidians		Na^+ L-type Ca^{2+}, T-type Ca^{2+}	Block & Moody, 1987 Cuomo et al., 2006 Dale et al., 1991 Gallo et al., 2013 Silvestre et al., 2009
		K^+ T-type Ca^{2+}	Izumi et al., 1999 Miller, 1975 Yoshida et al., 1994
Mollusks *Octopus Vulgaris*		L-type Ca^{2+}	Cuomo et al., 2005
Bivalves		K^+, Ca^{2+} L-type Ca^{2+}	Colas & Dube 1998 Dube, 1992 Guerrier et al., 1993 Leclerc et al., 2000 Moreau et al., 1990 Moreau et al., 1996 Tomkowiak et al., 1997

Fig. 1. Ion channel/current activities in gametes of invertebrates. *Types of ion currents/channels involved in the physiology of different maturation stages of invertebrate gametes. Oocytes are reported as GV immature stage and MI mature stage in ascidians, starfish and mollusks and MII mature stage in sea urchin. Spermatozoa are reported as immature spermatids and mature spermatozoa stages. In oocyte ion currents/channels are involved in either growth, maturation and fertilization. In spermatozoon ion currents/ channels are involved in either maturation, activation, motility, chemotaxis and acrosome reaction.*

The modifications during oocyte maturation in starfish species were associated to a decrease in membrane conductance, a depolarization of the membrane potential attributed to a change in Na+ and K+ conductance (Dale *et al.,* 1978; Miyazaki *et al.,* 1975a; Miyazaki *et al.,* 1975b; Moreau and Cheval 1976) and an hyperpolarization attributed to a reduction in chloride (Cl-) and Na+ permeability (Shen and Steinhardt 1976).

Following studies also confirmed that modifications of three major voltage-dependent currents underlined the maturation process in other two starfish species (*Henrician leviuscula* and *Asterina miniata)* (Simoncini and Moody 1990).

Ascidians are marine invertebrates whose oocytes are very suitable for the electrophysiological studies due to their extracellular coat that is very easy to removed, facilitating the access to the nude plasma membrane. Thanks to these characteristics, ascidian oocytes have been widely investigated. That ion currents in mature oocytes play a role in regulating early developmental processes in ascidians was early suggested by several authors at the end of 80'. In fact in *Boltenia villosa* metaphase I (MI) oocytes, three major voltage-dependent currents were described: a transient, inward Na+ current; a transient, inward Ca2+ current and an inwardly rectifying K+ current differently relating each one of these currents with following developmental events (Block and Moody 1987).

By using the whole-cell voltage clamp technique we characterized the electrical properties of immature oocytes of two ascidian species (Silvestre *et al.,* 2009; Tosti *et al.,* 2013). Although accurate electrophysiological characterizations in *Ciona intestinalis* first described the presence of L-type Ca2+ currents in the mature MI oocytes (Bosma and Moody 1990; Dale *et al.,* 1991), we showed a decline in the amplitude of these currents during the passage from GV to MI stage. In *Ciona intestinalis* oocyte plasma membrane, it was shown a higher L-type Ca2+ current activity at the GV stage in comparison to the following mature stage when Na+ currents start to appear (Cuomo *et al.,* 2006). The need for Ca2+ in the immature oocytes was also supported by the fact that these oocytes undergo a spontaneous maturation in presence of Ca2+. Later on, we better characterized the immature oocytes identifying three classes with different electrical features and demonstrated the gradual disappearance of high L-type Ca2+current activity in favor of appearance of Na+ currents that remained high throughout maturation up to the post-vitellogenic stage (Silvestre *et al.,* 2009). Of particular interest is the role of Na+ currents in the mature oocyte. In fact, we showed that by depriving of Na+ or by inhibiting the channels with tetrodotoxin the *in vitro* fertilization give rise to an anomalous 8-cell embryo

called rosette; furthermore the fertilization current in an environment Na+ free undergoes a reduction in the amplitude (Cuomo *et al.,* 2006). This clearly show that Na+ currents play a relevant role in the generation of first electrical events of fertilization and this is also in agreement with previous experiments (Tosti *et al.,* 2003) in which the inhibition of FC resulted in a long term effect on the normal embryo development. Nonetheless there is a general consensus for a role of L-type Ca2+ currents during oogenesis and maturation, surprisingly we recently showed, in the oocytes of the ascidian *Styela plicata*, an activity of T-type Ca2+ currents that underlie the nuclear maturation progress and oocyte growth by also inducing a progressive hyperpolarization of the membrane

Fig. 2. Ion channel/current activities in gametes of vertebrates. *Types of ion currents/channels involved in the physiology of different maturation stages of vertebrate gametes. Oocytes are reported as GV immature stage and MII mature stage. Spermatozoa are reported as immature spermatids and mature spermatozoa stages. In oocyte ion currents/channels are involved in either growth, maturation and fertilization. In spermatozoon ion currents/channels are involved in either maturation, activation, motility, chemotaxis and acrosome reaction.*

Vertebrates	Gamete	Currents/Channels	Refs.
Amphibians		H+, K+, Na+ , Cl-, L-type Ca2+, Ca2+ dependent Cl- , mechanosensitive cation	Barish, 1983 Barish & Baud, 1984 Baud, 1983 Ivorra & Morales, 1997 Jaffe & Cross, 1984 Lane et al., 1991 Morrill & Ziegler, 1980 Ouadid-Ahidouch, 1998 Schlichter, 1989, 1983 Wilkinson et al., 1998
Fishes		Ca2+	Cook et al., 1994 Ho & Suarez, 2001 Suarez and Ho, 2003
Rat		K+, Na+ Ca2+ , Cl-	Chan et al., 1997 Hagiwara & Kawa, 1984
Mouse		Ca2+ , L-type Ca2+ Aquaporins	Murnane et al., 1988 Murnane & DeFelice, 1993 Yamaji et al., 2011
		K+, Na+, Ca2+, Cl- L-type Ca2+, T-type Ca2+ Ca2+ CatSper, K+(SLO3)	Arnoult et al., 1996 Benoff, 2008 Darszon et al., 2011 Darszon, 2001 Escoffier et al., 2007 Florman et al., 1998 O'Toole et al., 2000 Patrat et al., 2000 Qi et al., 2007 Ren et al., 2001 Reyes et al., 1994 Santi et al., 1998 Son et al., 2002
Bovine		Ca2+, L-type Ca2+	Nichols, 2006 Tosti et al., 2000
Human		K+	De Felice et al., 1988 Du et al., 2010
		K+, Na+ Ca2+, Cl- Ca2+ dependent K+	Bai & Shi, 2001 Chan et al., 1997 Linares-Hernandez et al., 1998 Patrat et al., 2000 Rossato et al., 2001

potential. In particular, it was demonstrated a long term effect on the embryo development of these currents activity (Gallo *et al.,* 2013). This absolute novelty further support a role of Ca^{2+} currents during oocyte maturation.

The role of L-type Ca^{2+}currents during oocyte maturation has been also supported by numerous studies on the mollusks. In Spisula, Ruditapes and Crassostrea, a Ca^{2+} influx through voltage-gated channels is required for the occurrence of the germinal vesicle breakdown (GVBD) (Dube 1992; Leclerc *et al.,* 2000). These findings supported the fact that an early Ca^{2+} influx may be a prerequisite to allow nuclear maturation in either prophase I (Colas and Dube 1998) and MI-arrested oocytes of bivalves (Moreau *et al.,* 1996). The involvement of L-type Ca^{2+} currents in these processes was also demonstrated by other authors in either *Ruditapes philippinarum* (Guerrier *et al.,* 1993; Tomkowiak *et al.,* 1997) and *Octopus vulgaris* (Cuomo *et al.,* 2005) focusing on a possible role in the preparation of the plasma membrane to the interaction with the spermatozoon. In the latter, it is of interest the decrease of L-type Ca^{2+} activity during the growth and vitellogenesis of oocytes. Close to a predominant role of these currents, in the mollusk *Patella vulgata* a change in specific K^+ ion permeability due to the possible activation of stretch channels was also described during oocyte maturation (Moreau *et al.,* 1990).

Switching on vertebrates, a peculiar electrical property is that of amphibian oocytes which exhibit the unique presence of Cl^- channels in either immature and mature oocytes (Jaffe and Cross 1984; Schlichter 1989). In *Xenopus laevis*, other than a voltage-gated Na^+ current described by several authors (Bourinet *et al.,* 1992; Weber 1999) present during oocyte growth (Baud 1983), a more recent finding showed the presence of Cl^- currents and their ability to induce GVBD was demonstrated by means of molecular biology techniques (Reyes *et al.,* 2009). Furthermore the activity of a Ca^{2+} dependent Cl^- current in the immature oocytes was also characterized (Barish 1983). The coordination between Cl^- and Na^+ currents seems to modulate oocyte maturation in the frog *Rana pipiens* in which Cl^- channels disappear throughout maturation whereas the Na^+ currents remain active up to the full maturity of the oocyte (Baud 1983). In same species, an hormone-induced depolarization was also associated to a decrease in K^+ permeability (Morrill and Ziegler 1980). Together with these patterns, the fact that Cl^- currents are lost as the oocyte undergoes maturation (Schlichter 1983) suggested that Cl^- and Na^+ channels might play a regulatory role in maturation. However, the signal coming from L-type Ca^{2+} channels in the regulation of the maturation process was also described in pleurodeles (Ouadid-Ahidouch 1998).In the European frog *Rana esculenta*, together with Cl^- and Na^+ current activity, voltage-dependent K^+ current changes take place along-side oocyte maturation; these changes were considered relevant for a successful fertilization (Taglietti *et al.,* 1984). Subsequently, by accurate electrophysiological recording in *Rana perezi* three different types of Cl^- currents were characterized during oocyte growth (Ivorra and Morales 1997) relating them to a definite functional role in the maturation process. Along with the main currents described, the occurrence of a voltage-dependent hydrogen current in the Ambistoma immature oocytes was first reported (Barish and Baud 1984).

In the membrane of full-grown frog oocytes (Taglietti *et al.,* 1983), two kinds of voltage-dependent currents were described, the first was a specific and activated by hyperpolarizing steps more negative than -90 mV and the second was a cationic channel activated by depolarizing steps to potentials more positive than +40 mV. Since they were related to the inward and outward rectification of the intensity/voltage relationship, authors suggested that their function was to maintain the membrane potential at a constant value. An accurate electrophysiological characterization of the presence and properties of endogenous stretch-activated ion currents was performed in *Xenopus* mature oocytes, however no correlation with reproductive events was further suggested (Yang and Sachs 1990). Subsequently it was hypothesized a functional role of mechanosensitive cation-selective currents of *Xenopus* oocytes in the fertilization process (Lane *et al.,* 1991) since the inhibition of mechanically gated cation currents resulted in the reduction of the success of fertilization and the increased mortality in developing embryos (Wilkinson *et al.,* 1998). The involvement of cation channels in the physiology of *Xenopus* oocytes have been also confirmed by other authors who, studying the effect of maitotoxin, suggested the presence of a nonselective cation conductance possibly involved in changes of the membrane potential that occur during the early stages of fertilization (Bielfeld-Ackermann *et al.,* 1998). Recordings of Ca^{2+}-activated Cl^- currents and store-operated Ca^{2+} current activities in *Xenopus* were also suggested to play a role along with maturation promoting factor in the uncoupling between intracellular Ca^{2+} releasing and capacitative Ca^{2+} entry (Marin 2012).

In mammals, oocyte maturation is accompanied by relevant ion currents activities. In bovine immature oocytes the activity of two-pore K^+ currents participate to set the membrane potential and appear to contribute to the electrical activities of the plasma membrane during oocyte maturation (Hur *et al.,* 2009).

ATP-sensitive K^+ currents have been seen to regulate membrane potential affecting in the meantime, intracellular Ca^{2+} levels (Nichols 2006). These currents have been found in human, bovine and porcine oocytes at various maturational stages (Du *et al.,* 2010). The link established by these currents and intracellular metabolism, membrane excitability and intracellular Ca^{2+} signalling suggests a potential role to generate infertility due to the stress impact or metabolic diseases.

Voltage-dependent anion currents, which play a key role in mitochondrial metabolism, have been recently found on the membrane of porcine oocytes (Cassara *et al.,* 2009), they appear to be arranged as ring-like clusters of structures distributed on the cortical area at the GV stage, however this is not the case at the mature metaphase II (MII) stage oocytes. Either their localization or their absence at the end of maturation suggests a functional role during maturation and fertilization of mammalian oocytes.

Since plasma membrane is also the site of fluid movements between the inner and outer compartments of the cell, the water flow moves across the membrane either for simple diffusion or by means of ion channels (Verkman *et al.,* 1996). Aquaporins (AQPs) are a family of small membrane channel proteins that are expressed in plasma membranes of many cell types involving fluid transport (Carbrey and Agre 2009). In mouse oocytes, the *Aqp3* mRNA is expressed in immature oocytes and decreases after either *in vitro* or *in vivo* maturation (Jo *et al.,* 2011) suggesting a function of these channels in underlying correct maturation progress. In addition, experiments aimed to improve oocyte vitrification, confirmed a role of AQP3 in immature oocytes (Yamaji *et al.,* 2011).

The role of Ca^{2+} in oocyte maturation is still a matter of debate (Boni *et al.,* 2007). A wide body of literature deals on the role of

IP_3-induced Ca^{2+} release through IP_3 receptors during meiotic maturation, suggesting that this could be among the mechanisms underlying maturation of bovine oocytes. A minor role is instead attributed to Ca^{2+} release through ryanodine receptors, which are poorly expressed in bovine oocytes (He *et al.,* 1997). In the hamster and mouse, a series of spontaneous Ca^{2+} oscillations occur when oocyte are isolated from the follicle up to the GVBD stage (Carroll *et al.,* 1994; Fujiwara *et al.,* 1993), thereafter Ca^{2+} seems to not affect further meiotic progression. In bovine and pig, no Ca^{2+} oscillations occur during meiotic progression; although it has been shown that Ca^{2+} ionophore exposure induces meiosis resumption in oocytes arrested either at the GV (Wasserman and Masui 1975) or MII stage (Boni *et al.,* 2002).

Since extracellular Ca^{2+} is required for *in vitro* GVBD, meiosis progression (De Felici and Siracusa 1982; He *et al.,* 1997; Homa 1991) and the first meiotic division (Paleos and Powers 1981), Ca^{2+} ion transport through the plasma membrane seems to play a functional role in maturation. Electrophysiological and pharmacological characterization of ion currents underlying bovine oocyte maturation showed a predominant role of L-type Ca^{2+} currents (Tosti *et al.,* 2000), that however decrease throughout meiosis progression. Also in mouse oocytes, the activity of L-type Ca^{2+} currents have been demonstrated to underlay meiosis resumption (Murnane *et al.,* 1988) and to increases after puberty (Murnane and DeFelice 1993), suggesting a role for these currents in the acquisition of meiotic competence.

Recently, plasma membrane-resident Ca^{2+} channel proteins (STIM1 and Orai1) responsible for mediating Ca^{2+} entry after the mobilization of intracellular Ca^{2+}, have been identified in pig and mouse oocytes and their involvement in store-operated Ca^{2+} entry has also been demonstrated (Gomez-Fernandez *et al.,* 2009; Koh *et al.,* 2009; Yu *et al.,* 2009). Their up regulation has been shown to induce a dramatic increase in Ca^{2+} entry after intracellular store depletion. Interestingly, in pig Orai1 expression decreases during oocyte maturation (Wang *et al.,* 2012) whereas its down regulation inhibit Ca^{2+} influx after store depletion and the Ca^{2+} oscillations induced by the fertilizing sperm. On the other hand up regulation of Orai1 in the oocytes also modifies store-operated Ca^{2+} entry and plays an inhibitory effect on the fertilization Ca^{2+} signal, that results moreover in a long term negative effect on following embryo development.

In humans, the first studies on membrane potential have been performed by intracellular electrical recordings in immature oocytes collected by ovariectomy (Dolci *et al.,* 1985). Using the same electrophysiological technique, Feichtinger *et al.,* tested membrane potential variations in oocytes collected at different stages of maturation (Feichtinger *et al.,* 1988). Subsequently patch-clamp and whole-cell recordings were first applied in immature and mature oocytes. In the latter, the most frequently observed channel was a 60 picoSiemens non-inactivating, K^+ selective pore which was activated by depolarization (DeFelice *et al.,* 1988).

The spermatozoon

The spermatozoon is a highly specialized cell whose aim is to transport the genome into the oocyte and to trigger the oocyte into metabolic activation. In order to become competent for oocyte activation, the spermatozoon undergoes a series of changes in either its morphology or metabolism. Most of these processes are however accompanied by modifications in the electrical properties of the plasma membrane. Literature reports an increasing evidence that ion currents play a role in sperm physiology (Darszon *et al.,* 2001; Darszon *et al.,* 1999; Darszon *et al.,* 2008; Lishko *et al.,* 2012). By using an innovative technique of the patch-clamp applied to monolayers generated from a lipid vesicles and isolated sperm membranes, it was possible for the first time to record the ion current activity on the sea urchin sperm plasma membrane (Lievano *et al.,* 1985). Subsequently, this technique was applied to spermatozoa from other animal models reporting that main ion currents on the sperm plasma membrane were cations (K^+ and Ca^{2+}) and anion (Cl^-) currents (Chan *et al.,* 1997; Kirichok and Lishko 2006; Lishko *et al.,* 2013; Navarro *et al.,* 2008).

Immature male germ cells were specifically suitable for electrophysiological recordings since they are round and larger than the mature spermatozoa but also because they may express some of the proteins present on the plasma membrane of mature spermatozoon.

Earlier studies showed the presence K^+ and Ca^{2+} currents in rat spermatogenetic cells (Hagiwara and Kawa 1984), following investigation on rat spermatids suggested that Cl^-, K^+ and at a lesser extent Na^+ conductances were possible responsible for a negative membrane potential recorded (Reyes *et al.,* 1994). Subsequent studies highlighted a role for Cl^- conductance in spermatogenesis in *Caenorhabditis elegans* in fact a new inwardly rectifying Cl^- channel was characterized and put forward as an important candidate in the spermatid differentiation (Machaca *et al.,* 1996).

In mouse spermatogenetic cells, Santi *et al.,* documented a pH-dependent Ca^{2+} permeability and a series of K^+ selective currents correlating them with function of mature spermatozoa (Santi *et al.,* 1998). In particular, Ca^{2+} was correlated to the Ca^{2+} influx required for initiating the acrosome reaction (AR) at the time of first binding whereas K^+ currents were related to the hyperpolarization occurring for sperm fertilization competence (Felix *et al.,* 2002; Munoz-Garay *et al.,* 2001). The discovery of the expression of functional T-type Ca^{2+} channels during mouse spermatogenesis (Arnoult *et al.,* 1999; Son *et al.,* 2002) suggested an important role of these currents in the fertility block caused by contraceptive substances (Bai and Shi 2002).

The findings on immature sperm cells clearly showed an involvement of ion currents in either sperm production and function. In particular, the process that transforms the immature quiescent sperm in a cell competent for fertilization is the sperm activation, a process which is induced by the contact with the extracellular investments of the oocyte that clearly relies on the activity of ion currents.

Among the steps that allow sperm activation, chemotaxis is the attraction of motile sperm towards the oocyte after first chemical signals or binding of the gametes. Earlier studies on sea urchin spermatozoa showed that these cells are quiescent in the testis due to high K^+ concentration, pH, and oxygen tension, for review see (Tosti 1994). After spawning in the environment, changes in these parameters induced motility of spermatozoa. Subsequent studies demonstrated the latter involved sperm ion currents activity (Morisawa 1994). In fact, in echinoderms the compounds released from extracellular oocyte coat were able to induce ion fluxes and in turn a modulation of second messengers. Induction of sperm motility was shown to be essentially accompanied by K^+ currents modulation (Lee and Garbers 1986; Lievano *et al.,* 1985), whereas following studies showed that K^+ efflux at spawning generated a

cascade of other electrical events such as hyperpolarization of the membrane potential, Na^+/H^+ exchange, Na^+ influx, depolarization of membrane potential and at last Ca^{2+} currents, for review see (Darszon *et al.,* 2001). The finding of a cation current activity and sperm-activating peptides (SAPs) in the flagellum were then strictly related to the initial hyperpolarization associating it to the flagellar movements and to regulation of sperm trajectory respectively (Darszon *et al.,* 2008; Gauss *et al.,* 1998).

Also in other species sperm chemotaxis involving ion currents activity was described. In ascidians the initial observations of Miller (1975) were subsequently corroborated by other authors who showed the involvement of a K^+ permeability increase at contact point of sperm and oocyte followed by the hyperpolarization of the sperm plasma membrane which in turn mobilizes cAMP, a cAMP-dependent kinases that then activate sperm motility (Izumi *et al.,* 1999). Furthermore in ascidians the cascade of cAMP elevation were found to activate T- type Ca^{2+} currents (Yoshida *et al.,* 1994).

The role of K^+ currents was also demonstrated to regulate sperm motility in fishes, in particular due to the reverse of external osmolarity at the spawning. Following these initial events, an intracellular Ca^{2+} rise was also shown to trigger the initiation of sperm motility in marine and freshwater teleosts, salmonid and rainbow trout (Oda and Morisawa 1993; Takai and Morisawa 1995; Tanimoto *et al.,* 1994; Tanimoto and Morisawa 1988).

In mammals, the role of ion channels has been clearly demonstrated in chemotaxis, in fact some evidences indicating an involvement of intracellular Ca^{2+} release from stores in the mid-piece seems to mediate flagellar beating and, hence, chemotactic response.

The last basic event of sperm activation is the AR which is morphologically represented by the breakdown of the acrosomal vesicle that releasing its content allow the spermatozoon to exhibit a high fusible membrane (Florman *et al.,* 2008; Garbers 1989). AR is also induced at the contact with the extracellular layer of the oocyte, and many different compounds have been shown to be responsible for AR induction (Alves *et al.,* 1997; Ikadai and Hoshi 1981; Koyota *et al.,* 1997). The AR is recognized to be regulated by ion current activities and in particular a Ca^{2+} influx is an absolute requirement for AR in the sperm of all species studied so far (Darszon *et al.,* 2001; Darszon *et al.,* 1999; Florman *et al.,* 2008). However experimental evidences supported also a role of K^+ currents in AR induction (Schackmann *et al.*, 1978, Kazazoglou *et al.*, 1985, Yanagimachi, 1994).

The involvement of ion currents in AR was demonstrated in the sea urchin where an immediate Na^+ and Ca^{2+} influx and H^+ and K^+ efflux occur after the contact of oocyte jelly with a sperm receptor. These events also resulted in a change of membrane potential and an increase in pH due to a Na^+/H^+ exchange and the intracellular Ca^{2+} rise (Darszon *et al.,* 1999). Findings of two different Ca^{2+} currents along with Cl^- selective currents acting in AR were also reported (Guerrero and Darszon 1989). As in marine animals, in mammalian sperm the contact of the sperm receptor with the oocyte envelope (zona pellucida) causes elevation of intracellular Ca^{2+}, pH increase and a change in the membrane potential finally resulting in AR occurrence (Arnoult *et al.,* 1999; Darszon *et al.,* 2001; Florman *et al.,* 1998; Osman *et al.,* 1989).

An extensive literature on the presence of ion channels on the head of the mammalian spermatozoa, responsible for Ca^{2+} entry and intracellular Ca^{2+} rise, reports low and high voltage-activated, receptor-operated and store-operated Ca^{2+} currents (Benoff 1998;

Escoffier *et al.,* 2007). Furthermore T-type voltage gated Ca^{2+} currents were shown to play a peculiar role in the generation of AR (Darszon *et al.,* 1999; Darszon *et al.,* 2006; Florman *et al.,* 1998).

In human spermatozoa, along with the role of Na^+ currents activated by progesterone stimulus (Patrat *et al.,* 2000), the existence of voltage gated Ca^{2+} currents was demonstrated (Linares-Hernandez *et al.,* 1998), however their relation with the AR induction is still under investigation since the many indirect evidences reported (Darszon *et al.,* 2011; Jagannathan *et al.,* 2002). Among the mechanisms proposed for gating plasma membrane Ca^{2+} currents a capacitating Ca^{2+} entry mechanism play a relevant role. In fact in mouse spermatozoa, a Ca^{2+} influx generated by the depletion of Ca^{2+} stores was shown (O'Toole *et al.,* 2000). Following studies (Rossato *et al.,* 2001) suggested that Ca^{2+} stores depletion may be responsible for a double process: i) gating of Ca^{2+} activated K^+ currents, with K^+ efflux causing a hyperpolarization of membrane potential and ii) the capacitative gating of voltage-gated Ca^{2+} currents, with a subsequent depolarization of the plasma membrane. In the early 2000' it was reported that spermatozoa possess aspecific cation channel named CatSper (cation channel of spermatozoa), whose function seems to activate sperm motility. CatSper is a novel type of Ca^{2+} channel and the first member, CatSper1, was detected in mouse during searches for sequence homology to the voltage-gated Ca^{2+} channels (Ren *et al.,* 2001). Subsequently other 3 members of the family were discovered by means of molecular biology techniques CatSper 2, 3 and 4. These four CatSper proteins showed low identity with those found in all mammals examined, included human, the ascidian *Ciona intestinalis* and the sea urchin *Strongylocentrotus purpuratus*. Studies on the role and function of CatSper showed that it is one of the major Ca^{2+} conducting channel in spermatozoa (Darszon *et al.,* 2011) and that its role is strictly related to male infertility and in particular to sperm motility (Qi *et al.,* 2007; Singh and Rajender 2015; Williams *et al.,* 2015). This hypothesis was also corroborated by the fact that this current originated from the principal piece of the sperm flagellum where CatSper1 protein is located (Lishko and Kirichok 2010).

SLO3 is the high-conductance K^+ channel expressed only in mammalian testes, which is involved in multiple mechanisms of male fertility (Mannowetz *et al.,* 2013; Santi *et al.,* 2010) since it has been recognized to be responsible for capacitation-induced hyperpolarization and membrane hyperpolarization which is crucial for the AR. Many authors consider the SLO3 channel a putative candidate for a male contraceptive drug since Slo3 null mice are unable to perform progressive motility and to undergo the AR. A very recent report shows in mouse spermatozoa a possible functional connection between CatSper, SLO3, and transient receptor potential channel family members since the genetic deletion of either of those channels results in mouse male infertility (Chavez *et al.,* 2014).

An hypothesis that anions were involved in the mammalian AR (Morales *et al.,* 1993) was supported by electrophysiological studies that found different types of Cl^- channels with different conductance on the sperm head (Bai and Shi 2001) and by previous experimental evidence that mouse sperm Cl^- channels participate in the AR induced by the zona pellucida in mammals (Espinosa *et al.,* 1998; Sato *et al.,* 2000).

First electrical events at fertilization

Main steps of reproduction are the production of gametes (ga-

metogenesis), their maturation, reciprocal activation and at last their interaction that is widely recognized to be the key event of all the process.

Although it is different along the species studied, one of the earliest event of fertilization, is the electrical change of the oocyte plasma membrane. In the 50' a transient change in the membrane potential was described at fertilization and named as fertilization potential (Hiramoto 1959; Oddo and Esposito 1951). Nonetheless fertilization potential was described in oocytes of several marine animals (Dale *et al.,* 1978; Dale and de Santis 1981; David *et al.,* 1988; Lansman 1983; Sato *et al.,* 2000) only after the advent of the whole-cell voltage clamp technique, it was shown that these electrical modifications were due to a ion flux (the fertilization current-FC) across the plasma membrane. In 1984, Dale and De Felice in ascidian oocytes first characterized this current showing that FC is generated by the gating a peculiar population of ion channels in the newly fertilized oocyte (Dale 1994; Dale and De Felice 1984); (Tosti and Boni 2004) which in turn depolarized the membrane potential.

The characterization of the fertilization channels demonstrated that they are large, non-specific with a single conductance of 400 picoSiemens (Dale and De Felice 1984). In other ascidian species at fertilization a role of voltage-gated Ca^{2+} currents was shown in *Phallusia mammillata* (Goudeau and Goudeau 1993) and the appearance of a inward rectifying Cl^- current was described in *Boltenia villosa* (Coombs *et al.,* 1992).

In 1998, De Simone *et al.,* recorded in whole-cell voltage clamped sea urchin oocytes an inward Ca^{2+}-dependent FC demonstrating that it is driven by non-specific ion channels (De Simone *et al.,* 1998). In several amphibian species it was established that Cl^- channels in the oocytes are responsible for the fertilization potential (Jaffe and Schlichter 1985; Webb and Nuccitelli 1985), however a fertilization current which is at the basis of the fertilization potential in *Xenopus* oocytes was recorded only later on (Glahn and Nuccitelli 2003). This inward current resembled the ones recorded in the marine invertebrates. A careful characterization showed that the FC is underlied by the Ca^{2+}-activated Cl^- channels.

In mammals, first signs of electrical events at fertilization were a series of hyperpolarization that accompanied the sperm entry (Igusa *et al.,* 1983) showing the difference between mammalian and invertebrate oocytes. Subsequent studies aimed to record FC in mammalian oocytes (Gianaroli *et al.,* 1994; Miyazaki 1988; Tosti *et al.,* 2002) further highlighted such differences in the FC; in fact in human oocytes the FC is not the first event but it is generated by a Ca^{2+} release which in turn activate K^+ channels generating the outward current (Dale *et al.,* 1996).

The latter is also accompanied by a change in the oocyte membrane potential which transiently hyperpolarizes in all the mammalian oocytes. The function of these electrical events is still to be clarified in most of the cases, however Tosti *et al.,* associated the FC in the ascidian *Ciona intestinalis* with a long term effect on the embryo development (Tosti *et al.,* 2003) and a Ca^{2+}-activated outward current following cytosolic sperm factor injection was observed relating it to a signal for oocyte activation (Homa and Swann 1994).

Conclusion

In 1991, Neher and Sackman were awarded with the Nobel prize for the invention of the patch clamp technique. This proved the huge importance of the study of the ion channel activity that was initially applied to neurobiology (Parkinson), neuromuscular function and channelopathies (cystic fibrosis). The patch clamp technique has been subsequently used to clarify some mechanisms at the basis of the reproductive process. In this review we have provided an overview of the involvement of ion currents activities in the gametes physiology and the early events of fertilization, showing that ion currents are present from gametogenesis up to the embryo development and play a key role in the cell physiology and functionality of the related processes.

In the last 15 years, it has been claimed a decline in human fertility (Yoldemir and Oral 2012) and there is also an evidence of worrying decrease of some key species endangered by the environmental stress (Clulow *et al.,* 2014). So it is clearly evident the need to develop new techniques useful to improve *in vitro* maturation and fertilization; these are important reproductive technologies aimed to generates mature gametes capable of supporting embryo development and full development of the offspring.

The knowledge of the mechanisms governed by ion current activities may represent clinical and commercial incentive to improve the efficiency of these technologies in order to protect and increase the fertility of threatened animal species and human. Least but not last, ion current manipulation in gametes is a highly promising target to formulate new and soft non-hormonal contraceptive techniques.

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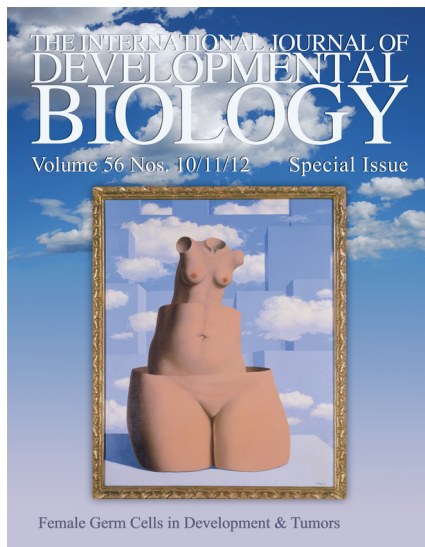
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