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Gamete activation: basic knowledge and clinical applications

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TABLE OF CONTENTS

BACKGROUND: The first clues to the process of gamete activation date back to nearly 60 years ago. The mutual activation of gametes is a crucial event during fertilization. In the testis and ovaries, spermatozoa and oocytes are in a state of meiotic and metabolic quiescence and require reciprocal signals in order to undergo functional changes that lead to competence for fertilization. First, the oocyte activates sperm by triggering motility, chemoattraction, binding and the acrosome reaction, culminating with the fusion of the two plasma membranes. At the end of this cascade of events, collectively known as sperm capacitation, sperm-induced oocyte activation occurs, generating electrical, morphological and metabolic modifications in the oocyte.

OBJECTIVE AND RATIONALE: The aim of this review is to provide the current state of knowledge regarding the entire process of gamete activation in selected specific animal models that have contributed to our understanding of fertilization in mammals, including humans. Here we describe in detail the reciprocal induction of the two activation processes, the molecules involved and the mechanisms of cell interaction and signal transduction that ultimately result in successful embryo development and creation of a new individual.

SEARCH METHODS: We carried out a literature survey with no restrictions on publication date (from the early 1950s to March 2016) using PubMed/Medline, Google Scholar and Web of Knowledge by utilizing common keywords applied in the field of fertilization and embryo development. We also screened the complete list of references published in the most recent research articles and relevant reviews published in English (both animal and human studies) on the topics investigated.

OUTCOMES: Literature on the principal animal models demonstrates that gamete activation is a pre-requisite for successful fertilization, and is a process common to all species studied to date. We provide a detailed description of the dramatic changes in gamete morphology and behavior, the regulatory molecules triggering gamete activation and the intracellular ions and second messengers involved in active metabolic pathways in different species. Recent scientific advances suggest that artificial gamete activation may represent a novel technique to improve human IVF outcomes, but this approach requires caution.

WIDER IMPLICATIONS: Although controversial, manipulation of gamete activation represents a promising tool for ameliorating the fertilization rate in assisted reproductive technologies. A better knowledge of mechanisms that transform the quiescent oocyte into a pluripotent cell may also provide new insights for the clinical use of stem cells.

Key words: oocyte / sperm / gamete activation / reproduction / fertilization / IVF / ICSI / artificial oocyte activation / assisted reproductive technology

Introduction

Reproduction is based upon a complex process of cell interaction and signal transduction that starts with the production of the gametes (spermatogenesis and oogenesis) and culminates with the formation of a zygote, the first cell of a new individual. Gametogenesis is underpinned by meiosis, the unique process of cell division that leads to the formation of haploid spermatozoa and oocytes. Gamete maturation occurs at the end of gametogenesis, with the formation of mature cells that are competent for fertilization, but in a state of meiotic and metabolic quiescence that can be reciprocally resumed by interaction with a partner. In fact, mutual activation of the gametes is an essential pre-requisite for fertilization, a process that involves numerous molecules, ions, cellular structures and metabolic pathways (Dale, 1983; Yanagimachi, 1994).

In the distant past, Spallanzani first realized that the contact between gametes was instrumental to achieve fertilization and initiate development (Magner, 1979).

The first clues that changes in sperm behavior and morphology were necessary to ensure oocyte fertilization were reported by Austin (1952), who defined the phenomenon observed as 'sperm capacitation'. Recent advances in knowledge about fertilization have shown that capacitation is a multistep process involving changes in sperm form and function that are induced by oocyte extracellular structures; the changes include motility, chemotaxis, binding, the acrosome reaction (AR) and fusion of the two plasma membranes (Fig. 1). Following oocyte-induced sperm activation, reciprocal sperm-induced oocyte activation occurs, with electrical, structural and metabolic modifications of the oocyte that result in successful fertilization and the triggering of embryo development (Fig. 2).

In this review, we describe the events and the molecular basis of sperm–oocyte interaction in the principal animal models used for experimental research and in the human. Manipulation of gamete activation to improve human-assisted reproductive technology (ART) is also discussed.

Methods

This review describes the events surrounding sperm–oocyte interaction and the structural and functional changes that gametes undergo before successful fertilization and the triggering of embryo development. Clinical and scientific evidence on the use of artificial gamete activation to improve human ART is also discussed. We performed an electronic search of PubMed, Google Scholar and Web of Knowledge for full texts and abstracts published before 18 March 2016. We used the following combinations of key words:

Gamete/sperm/oocyte versus: activation/fertilization/embryo development.

Gamete/sperm/oocyte versus: quiescence/motility/chemotaxis/binding/AR/fusion. Gamete/sperm/oocyte versus ion currents/calcium/meiosis/activators/sperm factor (SF).

Gamete/sperm/oocyte versus artificial activation/ART/IVF/ICSI.

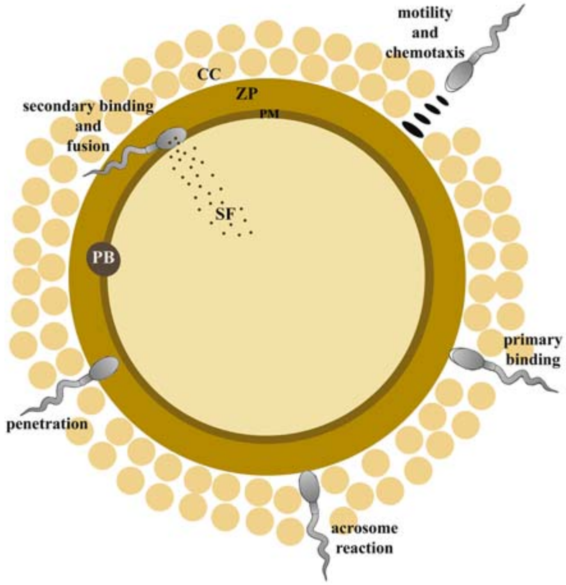
Articles were restricted to English language full-text articles.

Sperm activation

Quiescence

Sperm within the testis are maintained in a quiescent state. Research in marine animals from 1948 onwards led to the hypothesis that factors such as ion concentration, pH and osmolality were responsible for this metabolic suppression (Rothschild, 1948). To confirm this hypothesis, many authors submitted sperm of species with external fertilization to changes in environmental cues, showing that sperm gained the ability to fertilize with a reversal of potassium and sodium concentrations and changes in pH (Tosti, 1994). In mammals, testicular sperm are essentially motionless and not capable of fertilizing oocytes. When sperm leave the testis, they are coated with several macromolecules that are gradually lost as they traverse the tubules of the epididymis, thus acquiring the ability to move. This process, known as epididymal maturation, is underpinned by reversal of ion concentrations and pH, and by inorganic and organic factors present in the epididymal tubular fluid (Verma, 2001). Interestingly, mouse studies comparing ejaculated and epididymal sperm treated under identical standard conditions for *in vitro* capacitation showed that the two types of sperm display different swimming characteristics, and ejaculated sperm are more efficient at penetrating the cumulus oophorus, demonstrating the advantage gained from exposure to accessory gland secretions (Honggang *et al.*, 2015).

However, in order to become fully competent for fertilization, after ejaculation mammalian sperm need a period of residence in the female reproductive tract, where further molecular, biochemical and physiological changes occur (Salicioni *et al.*, 2007).

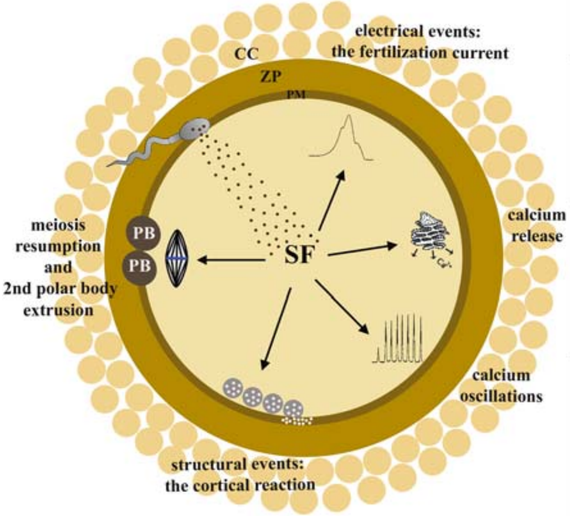


EVENT	COMPONENTS INVOLVED	MAIN REFERENCES
Motility	cAMP PKA ATP	Tosti, 1994 Aitken, 2000 Ferramosca and Zara, 2014
Chemotaxis	CAMP cGMP NO potassium/calcium channels CRISP Catsper	Eisenbach, 1999 Kirkman-Brown et al., 2003 Miraglia et al., 2007 Jikeli et al., 2015 Yoshida and Yoshida, 2011 Ernesto et al., 2015
Primary binding	Bindin α-L-fucosidase SPAM1/HYAL5 ZP1-2-3 PH-20 spermadhesin/pro/acrosin/sp17/sp56/sp38 zonadhesin/glutathione-S-transferase/ADAM3 lactoferrin	Vacquier, 2012 Matsumoto et al., 2002 Yoon et al., 2014 Gupta, 2015 Gmachl et al., 1993 Tanphaichitr et al., 2015 Zumoffen et al., 2013
Acrosome reaction	SNARES acrosomal enzymes l-amino acid oxidase calcium channels/Catsper cAMP/PKA	Kierszenbaum, 2000 Harper et al., 2008 Houston et al., 2015 Stamboulian et al., 2004 Ren et al., 2001 Tateno et al., 2013
Secondary binding and Penetration	ZP2 proacrosin/acrosin PH-20 zonadhesin sp56 SPESP	Tsubamoto et al., 1996 Töpfer-Petersen and Cechová,1990 Cherr et al., 1996 Tardif et al., 2010 Muro et al., 2012 Fujihara et al., 2010
Fusion	PH30 ADAMs DE/CRISP CD9 Izumo/Juno	Primakoff et al., 1987 Stein et al., 2004 Cohen et al., 2011 Da Ros et al., 2015 Jankovičová et al., 2015 Bianchi and Wright, 2014 Klinovska et al., 2014

Figure 1 Oocyte-induced sperm activation.
Left panel: image representing the events occurring during oocyte-induced sperm activation: initially, chemical signals from the oocyte extracellular membranes rescue sperm from a quiescent state by triggering motility and attracting the sperm toward the oocyte itself. The primary binding, which occurs between ligands and receptors present on their external sides, induces the AR, which renders the sperm plasma membrane highly fusible. This event allows penetration through the ZP and triggers fusion of the two PM. Upon fusion, sperm start to release the SF into the oocyte cytoplasm. Right panel: table reporting the event, the components involved and relevant references. PM, plasma membrane; ZP, zona pellucida; CC, cumulus cells; SF, sperm factor; PB, polar body; cAMP, 3′-,5′-cyclic adenosine monophosphate; PKA, protein kinase A; cGMP, cyclic guanosine monophosphate; NO, nitric oxide; CRISP, cysteine-rich secretory protein; SPAM1 and HYAL5, hyaluronidases; PH-20, glycosylphosphatidylinositol-anchored protein; ADAM3, metalloproteinases; SNARES, soluble NSF-attachment protein receptors; SPESP, sperm equatorial segment protein; PH-30, fertilin; DE, epididymal protein; CD9, member of the tetraspanins protein family; AR, acrosome reaction; sp, sperm surface protein.

Motility

Although initiation of motility was observed immediately after the reversal of environmental conditions, little is known about the molecular mechanisms that control this process. Since sea urchin sperm release acid after spawning, Ohtake (1976) first focused on the role of intracellular pH, showing an association between oxygen consumption and a pH increase. Other studies supported this hypothesis, demonstrating that low pH acted by inhibiting ATP hydrolysis. ATP hydrolysis provides energy by mitochondrial oxidation of fatty acids, and this is promptly initiated when sperm cytoplasm is alkalinized (Shapiro *et al.*, 1985). The increase in intracellular pH was reported to induce sperm movement in echinoderms (Christen *et al.*, 1982) and mammals (Babcock *et al.*, 1983), possibly by a mechanism related to the activation of dynein, the molecular component of the axoneme which is the motor for sperm tail flagellar motility (Nakajima *et al.*, 2005). Along with the increase in pH, a variety of mechanisms have been proposed to explain the activation of mammalian sperm motility at ejaculation. One of the major factors involved in the regulation of sperm movement is an increase in intracellular 3′-,5′-cyclic adenosine monophosphate (cAMP) (Jones and Murdoch, 1996), whose changes seem to mediate the regulatory proteins associated with the axoneme through a protein kinase A (PKA) regulatory pathway (Aitken, 2000). In particular, phosphorylation of axonemal proteins plays a crucial role in the regulation of motility and cAMP-dependent protein phosphorylation has been proposed as a central mechanism for triggering motility (Carr and Acott, 1989).



EVENT	COMPONENTS INVOLVED	MAIN REFERENCES
Sperm factor release	PLC ζ /γ PAWP PIP2	Tosti and Menezo, 2010 Dale et al., 2010 Kashir et al., 2014 Amdani et al., 2015a Swann et al., 2004 Nomikos et al., 2015
Fertilization current	fertilization channels sodium /calcium currents calcium-activated potassium channels calcium-activated chloride channels	Dale and De Felice, 1984 Gianaroli et al., 1994 Homa and Swann, 1994 Tosti et al., 2002 Glahn and Nuccitelli, 2003
Calcium release and oscillations	calcium ions calcium stores STIM/ORAI/SERCA IP_3/cADPr/NAADP TPCs	Whitaker, 2006 Kashir et al., 2013 Krauchunas and Wolfner, 2013 Bernhardt et al., 2015 Willmott et al., 1996 Ramos and Wessel, 2013
Structural modifications	CG calcium SFE1/SFE9/proteoliaisin/rendezvin PKC SNAREs α-SNAP/ NSF ovastacin	Wong and Wessel, 2004 Takeo et al., 2014 Oulhen et al., 2013 Sun, 2003 Liu, 2011 De Paola et al., 2015 Quesada et al., 2004
Meiosis resumption	MPF/MAPKZP2 cyclin B PKC/CAMKII/calcineurin EMI2/ERP Mos Cdk1/Cdc2 zinc mRNA polyadenylation	Madgwick and Jones, 2007 Suzuki et al., 2010 Backs et al., 2010 Von Stetina and Orr-Weaver, 2011 Duprè et al., 2011 Krauchunas and Wolfner, 2013 Que et al., 2015 Charlesworth et al., 2013

Figure 2 Sperm-induced oocyte activation.

Left panel: image representing events occurring during sperm-induced oocyte activation: upon release of the SF, electrical modification of oocyte plasma membrane properties generates an outward (in mammals) or inward ion current (non-mammals). Release of calcium from the intracellular stores generates calcium oscillations. Physical changes of the oocyte occur by release of CG contents, and finally meiosis is resumed, allowing completion of the cell cycle, extrusion of the polar body and triggering of zygote formation. Right panel: table reporting the event, the components involved and relevant references. PLCζ, phospholipase C; PAWP, postacrosomal sheath WW domain-binding protein; PIP_2, phosphatidylinositol (4,5)-bisphosphate; IP_3, inositol 1,4,5-trisphosphate; cADPr, cyclic adenosine diphosphoribose; NAADP, nicotinic acid adenine dinucleotide phosphate; TPCs, two-pore channels; SFE1, SFE9, structural matrix proteins; PKC, protein kinase C; SNAP, *N*-ethylmaleimide-sensitive factor attachment protein alpha; NSF, *N*-ethilmaleimide sensitive factor; MPF, maturation promoting factor; MAPK, mitogen-activated protein kinase; CAMKII, calcium calmodulin-dependent protein kinase; EMI/ERP, early mitotic inhibitors; Mos, serine/threonine kinase; Cdk1, cyclin-dependent kinase; CG, cortical granule; SF, sperm factor.

The maintenance of sperm motility is also dependent on the presence of calcium, although this appears not to be a physiological factor associated with sperm quiescence, but is instead associated with an increase in cAMP content (Aitken, 2000). Recently, direct recordings from sperm of different mammalian species, including humans, revealed that sperm motility is regulated by ion homeostasis, which in turn is under the direct control of ion channels, and in particular a potassium-induced hyperpolarization of the plasma membrane (Miller *et al.*, 2015). In mammals, although ejaculated sperm are motile their ability to fertilize an oocyte is reduced. This may definitely occur after the removal of inhibitory factors such as surface-attached glycoproteins, seminal plasma proteins and depletion of membrane cholesterol. This final state of activated sperm is known as hyperactivation, and is a high energy phase of vigorous flagellar movement and swimming capacity (Lishko *et al.*, 2012). This form of motility may be induced close to the time of ovulation and seems to be associated with a requirement for enhanced progressive motility through the oviduct. It is clear that sperm motility is a process that responds to signals from the external environment, and this also includes products secreted by the oocyte. In non-mammalian species, oocyte-released factors, such as sperm motility initiation factor and herring sperm activating peptide, modulate sperm motility, whereas in mammals progesterone and some unidentified components of cumulus cells have been recognized as responsible for sperm hyperactivation (Quill and Garbers, 2002).

In order to undergo activation, sperm require an adequate supply of energy from intracellular biochemical events such as oxidation of energy substrates, phosphorylation of proteins and conversion of chemical energy into mechanical energy. Glycolysis and mitochondrial oxidative phosphorylation are the major metabolic pathways that generate this source of energy, in the form of ATP (Ferramosca and Zara, 2014).

A peculiar modulation of sperm behavior is also triggered by different compartments of the female tract into which they are released, especially in terms of protein composition, glycosylation and hydration of the cervical mucus (Druart, 2012). Furthermore, micronutrients contained in the seminal plasma may also play a significant role in inducing sperm motility (Macchia *et al.*, 2010).

Chemotaxis

Once motile, sperm swim toward the oocyte in response to chemical concentration gradients. Indeed, it is difficult to separate motility activation from chemotaxis, since factors that activate motility may be the same as those responsible for directing sperm toward the oocyte. To demonstrate a true chemoattraction, the spermatozoon must change direction and modify the longitudinal waveform of the flagellum in response to an increased concentration of factors released by the oocyte or its extracellular coats (Miller, 1985). In species with external fertilization, chemotaxis is of crucial importance due to the high dispersion of gametes in the external environment. In the 1980s, many small peptides were isolated and characterized in the so-called water egg of marine invertebrates. These peptides, in addition to stimulating sperm motility and respiration, exerted a clear species-specific chemoattractivity (Ward and Kopf, 1993). In particular, resact, a small peptide isolated from the oocyte jelly layer of the sea urchin, induced a calcium-dependent swimming pattern that reoriented from a circular to a straight trajectory. Guanylate cyclase appeared to be the receptor for resact, and in fact this process is also accompanied by a transient activation of enzyme activity and a burst of cyclic guanosine monophosphate (cGMP) synthesis. It was hypothesized that cGMP hyperpolarizes the sperm cell due to efflux of potassium ions via selective ion channels, followed by a modulation of intracellular calcium levels that initiates straight swimming (Eisenbach, 1999; Kirkman-Brown *et al.*, 2003; Hildebrand and Kaupp, 2005). Cyclic nucleotides have been implicated as mediators of changes in both motility and chemotaxis; cAMP-dependent protein phosphorylation has been recently shown to mediate sperm movement in the ascidians, directed by a sperm activating and attracting factor (Shiba and Inaba, 2014). This also seems to be the case for mammalian and human sperm, where cAMP and cGMP are involved in ovarian progesterone-mediated sperm attraction (Teves *et al.*, 2009; Gakamsky *et al.*, 2009; Chang and Suarez, 2010). In human sperm, nitric acid was suggested to elicit a chemoattractant effect through a signal transduction involving the synthesis of cGMP and the activation of cGMP-dependent protein kinases (Miraglia *et al.*, 2007).

Sea urchin sperm show a mechanism of cGMP-induced hyperpolarization of membrane potential, via potassium efflux through cGMP-activated potassium channels that in turn gate calcium channels. Calcium entry is an important factor in regulating sperm chemotactic behavior, and this is likely to be a feature that is common to all species (Strünker *et al.*, 2006; Yoshida and Yoshida, 2011). A recent sophisticated study using holographic microscopy and optochemical techniques confirmed previous findings, and a model has been proposed to suggest that cGMP acts as a transducer of messages between sperm and chemoattractants. In particular, cGMP degradation and inactivation of the guanylyl cyclase receptor appear to control recovery from hyperpolarization, and thus the amplitude and timing of calcium responses (Jikeli *et al.*, 2015). In mammals, chemical communication between gametes seems to be less important, since sperm are deposited in the female genital tract and it is feasible that some sperm may reach the oocyte in a random manner. Over the last few decades, there has been evidence to indicate that mammalian sperm are able to respond to chemoattractants secreted from the oocyte, the extracellular coats and their surrounding environment. In some mammals, sperm stored in the caudal isthmus have a reduced motility that is resumed after ovulation (Ward and Kopf, 1993). In women, sperm are stored in the cervix; however, the sperm:oocyte ratio at fertilization ranges from 10:1 to 1:1, suggesting that a possible selection of sperm occurs in the female genital tract. In fact, when ovulation occurs, some sperm stored in the reservoir resume motility and travel up to the fertilization site within a few minutes. It is of interest that these sperm are able to detach from the epithelium when released from the storage site, supporting the idea that they receive signals from the oviduct (Eisenbach, 1999 and references therein). Follicular fluid (FF) contains secretions from the oocyte and the cumulus oophorus, and earlier studies showing that only FF from fertilizable oocytes acts as a chemoattractant (Ralt *et al.*, 1994) were further supported by Sun and colleagues (2005), who revealed that the two real chemoattractant sources are the mature oocyte and the surrounding cumulus cells. Many substances have been proposed as potential attractants in FF, including peptides, heparin, hormones and oxytocin, but only progesterone has been clearly demonstrated as causing sperm accumulation in humans, and as being the main, if not the sole, chemoattractant secreted by human cumulus cells (Oren-Benaroya *et al.*, 2008; Armon and Eisenbach, 2011). Notably, although the nature of the progesterone receptor remains to be elucidated, an involvement of CatSper, a pH-dependent calcium channel of the sperm flagellum, appears to be the mechanism that links progesterone and calcium elevation in the chemotactic process (Lishko *et al.*, 2011). Evidence was also provided that CRISP1, a sperm protein expressed by the cumulus cells that surround the oocyte, stimulates sperm orientation by modulating sperm hyperactivation and showing the ability to regulate CatSper (Ernesto *et al.*, 2015). Interestingly, an olfactory receptor whose function seems to be critical for chemotaxis was identified, cloned and shown to be functionally expressed in human testicular sperm (Spehr *et al.*, 2003). Finally, two recent findings showed an oocyte-derived chemotactic activity associated with a hydrophobic nonpeptide molecule in human sperm (Armon *et al.*, 2014) and an association between follicle rupture and uterine contractions with the success of human *in vivo* insemination suggests the existence of possible chemoattractive substances in the female tract (Blasco *et al.*, 2014).

Primary binding

Primary binding is the first physical contact of gametes that involves species-specific recognition of structures and molecules (Bi *et al.*, 2002). Adhesion between gametes follows a sequence of events that involves low- and high-affinity binding sites. As a general rule, a molecule located on the sperm would recognize and bind to a complementary molecule located on the oocyte coat. The major structures involved in gamete binding are similar in sperm of different species, but are different in the oocytes; in fact, although they share the same functions, the extracellular coats responsible for initial adhesion with sperm are of a different composition and name: the vitelline layer in the sea urchin, chorion in the ascidians and zona pellucida (ZP) in most mammals. Species-specificity refers mainly to different molecular events, such as enzyme–substrate and ligand–receptor interactions. Although it is feasible that acrosome-reacted sperm should be unable to bind to the extracellular coat, exceptions are reported in sea urchin and mouse. In the sea urchin, initial adhesion occurs between the vitelline layer and bindin, a molecule that is revealed after the AR; this peculiar behavior was attributed to an AR-inducing activity of the oocyte jelly layer (Vacquier, 2012). Similarly, mouse sperm were shown to initiate their AR before contact with the ZP, and are still able to cross the ZP and fertilize the oocyte after undergoing the AR (Jin *et al.*, 2011; Inoue *et al.*, 2011). In ascidians, a general substrate–enzyme mechanism mediated by a sperm α-L-fucosidase and the complementary-L-fucosyl residues of glycoproteins on the oocyte chorion was first hypothesized in *Ciona intestinalis* (Hoshi, 1986), and subsequently demonstrated in *Halocynthia roretzi* (Matsumoto *et al.*, 2002). Although this is considered to be the prevailing molecular mechanism, it is likely that molecules other than glycosidases participate in the initial sperm–oocyte adhesion. Trypsin-like acrosin and spermosin proteases have been proposed to be involved in this process, suggesting that a proteasome system enables spermatozoa to penetrate the chorion or participate in the process as sperm-related 'moveable' binding proteins (Sawada *et al.*, 2014). A conserved mechanism of gamete recognition in mammals is based on carbohydrate–protein interaction between sperm and oocyte. Some oligosaccharides arranged on the outer structure of the oocyte envelope are recognized by complementary proteinaceous receptors on the sperm. Sperm hyaluronidases are believed to play an essential role in mammalian fertilization, and sperm-specific SPAM1 and HYAL5 hyaluronidase were suggested to be involved in sperm–ZP binding in the mouse. However, recent studies show that hyaluronidases are not required for fertilization, arguing against the role of oligosaccharides in gamete recognition mechanisms (Zhou *et al.*, 2012; Yoon *et al.*, 2014).

The mammalian ZP is an elastic structure formed by a network of fibrils and filaments. The absence of ZP production renders females infertile, whereas removing the ZP from virgin oocytes eliminates species-specific recognition *in vitro* (Wassarman and Litscher, 2008, 2012).

The functional region known as the ZP domain is composed of three glycoproteins (ZP1, ZP2 and ZP3) that are synthesized and secreted during oocyte growth and whose structure and function differ according to the species (Töpfer-Petersen *et al.*, 2000). In particular, human ZP contains an additional glycoprotein, ZP4, which is a paralogue of ZP1. At the beginning of the 1980s, ZP3 was identified in mouse as the 'primary receptor' responsible for species-specific sperm recognition and adhesion (Bleil and Wassarman, 1980). Subsequent studies aimed at elucidating multiple functions of ZP3 showed that only the glycoprotein from unfertilized oocytes allows sperm to undergo and complete AR exocytosis (Florman and Storey, 1982; Bleil and Wassarman, 1983; Wassarman and Litscher, 2008).

Many authors have suggested that ZP1, ZP3 and ZP4 bind to capacitated human sperm and induce the AR, whereas ZP2 binds only to acrosome-reacted spermatozoa, and thus may be considered a secondary sperm receptor (Gupta *et al.*, 2012; Gupta, 2015), however, this has not been validated by *in vivo* studies, and is still subject to question.

The search for the counterpart of ZP3 in mouse led to the identification of a galactosyltransferase (GalTase) on the sperm surface, which is able to recognize ZP3 oligosaccharides and then trigger the AR, relying on a G-protein-mediated process (Florman and Wassarman, 1985; Miller *et al.*, 1992). A surface GalTase was also expressed in various mammalian species and was found in the appropriate location to bind to the ZP, suggesting that the mouse mechanism of sperm–oocyte binding is conserved among species (Larson and Miller, 1997).

In humans, a homolog to the PH-20 glycoprotein present on the head of guinea pig sperm was also identified as playing a role in binding sperm to the oocyte ZP through hyaluronidase activity (Gmachl *et al.*, 1993). Subsequent studies that focused on identifying novel gamete receptors have characterized SED1, a sperm protein that is required for sperm adhesion to the oocyte coat; this is secreted by the epididymal epithelium and coats sperm during progression through the epididymis. Related studies identified a second novel ligand on the oocyte coat, originating from oviduct secretions, which acts as a ZP3-independent component. This is a high-molecular-weight wheat-germ-agglutinin glycoprotein that appears to participate in primary sperm binding (Ensslin *et al.*, 2007). Although the central role of ZP3 as a sperm-binding partner and AR inducer is extensively supported in the literature, the nature of the proteins that bind sperm is still controversial.

Another set of possible ZP receptors exists along with the glycoenzymes including a large array of sperm surface proteins such as spermadhesin and pro/acrosin, sp17, sp56, sp38, zonadhesin and spermadhesins, glutathione (GSH)-S-transferase and ADAM3 (Yamaguchi *et al.*, 2006; for review, see Tanphaichitr *et al.*, 2015). In mice, ZP3 has been proposed to act as primary sperm receptor and the primary inducer of the AR, whereas in human, the AR appears to be mediated by either ZP1, ZP3 or ZP4 that have been shown to bind to the capacitated sperm (Gupta, 2015).

In contrast to the concept that ZP2 is a secondary receptor involved in binding between acrosome-reacted sperm and ZP (Florman and Wassarman, 1985), a mechanism by which mouse and human sperm bind oocyte ZP2 has recently been proposed as an alternative to ZP3 (Avella *et al.*, 2014).

The oviductal environment and its secretions also play a critical role in transport and interaction of male and female gametes. Recent findings report the expression of lactoferrin, a human oviductal protein able to inhibit gamete interaction *in vitro* and possibly involved in the regulation of the reproductive process via a role in polyspermy prevention (Zumoffen *et al.*, 2013). The same authors later demonstrated that lactoferrin causes a decrease in sperm α-D-mannose-binding sites and an increase in tyrosine phosphorylation of sperm proteins, suggesting that this protein is able to modulate parameters of sperm function (Zumoffen *et al.*, 2015).

Acrosome reaction

The AR is an exocytotic calcium-dependent process, considered to be the major pre-requisite for sperm penetration through the oocyte coats. The acrosome is a secretory vesicle located on the tip of the sperm head whose structure is variable among species. As a general view, the acrosome has an outer membrane that lies beneath the sperm plasma membrane and an inner membrane that overlays the nuclear membrane. The two membranes are continuous, but appear to be parallel, enclosing a space containing hydrolytic enzymes. Acrosome exocytosis is a calcium-dependent event based on multiple fusions between the outer membrane and the closely apposed plasma membrane, as well as the interaction of a specific pair of proteins, called SNAREs (soluble NSF-attachment protein receptors) (Kierszenbaum, 2000). The patches formed allow the highly fusible inner acrosomal membrane to be exposed, with dispersion of acrosomal enzymes (including hyaluronidase and trypsin-like proteases) that are crucial for digestion and penetration of the oocyte extracellular matrix (Harper *et al.*, 2008). In almost all species studied, no morphological modifications are evident at the AR, with the exception of echinoderms where polymerization of G-actin into F-actin induces the extension of an elongated structure known as the acrosomal process. The AR is induced after the first binding with adhesion between ligands and receptors, but the nature of the AR inducers remains an unresolved question. In the sea urchin, fucose sulfate glycoconjugates and polymers, components of the egg jelly coat, were shown to play a role in stimulating the AR (Decker *et al.*, 1976; Mengerink *et al.*, 2000). Molecular characterization of acrosome reaction-inducing substance recently confirmed the role of this highly sulfated glycoprotein as an AR-inducing substance in starfish (Hoshi *et al.*, 2012).

In mammals, two main questions arise: where and how the AR takes place (Yanagimachi, 2011; Buffone *et al.*, 2014). While many studies corroborate the role of solubilized ZP or purified ZP3 as main inducers of exocytosis (Gupta and Bhandari, 2010), hamster and rabbit sperm seem to initiate the AR while advancing through the cumulus cells that surround the ZP. In fact, the response of sperm to progesterone (Roldan *et al.*, 1994), which is one of the major secretory products from the cumulus cells, reinforces this hypothesis. In support of the idea that the ZP is not sufficient to induce the AR (Baibakov *et al.*, 2007), recent advanced investigations that distinguished fertilizing sperm from their non-fertilizing counterparts revealed that mouse sperm had already undergone the AR when first observed in the cumulus (Jin *et al.*, 2011), confirming earlier studies on guinea pig sperm (Huang *et al.*, 1981). The timing of the AR is at present matter of debate, with controversial studies published; a reconsideration of a zona-induced AR has been recently reviewed (Okabe, 2014).

As previously mentioned, the AR is a process strictly sustained by an increase in cytoplasmic calcium concentration that precedes exocytosis, essential for the activation of phospholipases and for fusion of the outer acrosomal membrane with the plasma membrane (Florman and Ducibella, 2006; Florman *et al.*, 2008). Recent findings in human sperm also report: the existence of L-amino acid oxidase with a potential role in driving the regulation of sperm capacitation and acrosomal exocytosis either in the presence or absence of progesterone (Houston *et al.*, 2015); the activation of an adenylyl cyclase downstream in opening store-operated calcium channels during the swelling process (Sosa *et al.*, 2016).

In mammals, two sperm signaling pathways underpin exocytosis of the acrosomal contents (Primakoff and Myles, 2002). After initial binding, a GTP-binding protein and phospholipase C (PLC) are activated, followed by elevation of intracytoplasmic calcium. Alternatively, a transient influx of calcium takes place through low-voltage T-type channels and their subtypes Cav3.2 (Stamboulian *et al.*, 2004), a calcium store depletion pathway (O'Toole *et al.*, 2000), whereas additional calcium entry occurs through the activation of Trp family calcium channels (Jungnickel *et al.*, 2001).

Together with the above-mentioned ion channels, sperm have a number of calcium-conducting channels, including high-voltage L-type, cyclic nucleotide-gated channels and the CatSper channels. CatSper was first described in mouse sperm tails (Ren *et al.*, 2001) and identified as playing a role in sperm motility. Today, it is known that CatSper orthologs are present in all mammalian (including human) sperm, exerting a sort of 'multitask' function in male fertility (Singh and Rajender, 2015).

In addition to ion channels, other cell signaling pathways such as changes in sperm membrane potential participate in calcium elevation. In particular, strong membrane hyperpolarization due to increased activity of potassium channels and an associated decreased activity of sodium channels appear to regulate the ability of sperm to generate transient calcium elevation (Arnoult *et al.*, 1999; Rossato *et al.*, 2001) and prepare sperm for the AR (De La Vega-Beltran *et al.*, 2012). An elevation of intracellular pH driven by a G protein-dependent pathway was also reported to be among the initial responses to first binding (Arnoult *et al.*, 1996). This event seems to be associated with adenylate cyclase activation and consequent cAMP production (López-Úbeda and Matás, 2015). The subsequent activation of a cAMP/PKA pathway leading to protein tyrosine phosphorylation is also one of the debated molecular changes associated with acrosome exocytosis (Tateno *et al.*, 2013).

Different phospholipase isozymes are present in the sperm head, and these may contribute to the ZP-induced AR via hydrolysis of phospholipids. These products are involved in the final stages of membrane fusion, or may represent substrates for the generation of downstream metabolites such as inositol triphosphate (IP_3) and diacylglycerol (DAG) (Roldan and Shi, 2007). These data are consistent with the presence of IP_3 and ryanodine receptor ion channels devoted to calcium release on the sperm head.

Secondary binding and penetration

Once acrosome reacted, sperm proceed to oocyte matrix penetration, which occurs by enzymatic digestion and vigorous movement of the sperm tail. During penetration, the sperm cell must maintain its adherence to the oocyte coat via transient secondary binding supported by new different ligands and receptors. In mouse, earlier studies postulated that ZP2 acts as a secondary receptor for sperm, involving a sperm trypsin-like proteinase as a counterpart (Bleil *et al.*, 1988). Evidence was later presented to suggest that proacrosin/acrosin is one of the complementary binding proteins to mouse ZP2 on sperm, apparently acting as a bridge between the newly exposed inner acrosomal membrane and the zona matrix. In fact, it was shown that porcine proacrosin recognizes ZP2 (Tsubamoto *et al.*, 1996) and that proacrosin, along with participation in secondary binding, is converted to acrosin and plays an essential role in zona penetration (Töpfer-Petersen and Cechová, 1990). Interestingly, this mechanism is identical to that previously described for sea urchin bindin–vitelline layer interactions (Howes *et al.*, 2001). In pig, the secondary binding ligand for sperm seems to be ZP1 translocated from the equatorial segment to the posterior, suggesting that it assists sperm in zona penetration. This mechanism seems to be conserved, since recombinant porcine ZP1 was shown to also bind to the equatorial region of the sperm head in five different mammalian species (Tsubamoto *et al.*, 1996). Several other proteins have been characterized as sperm–oocyte matrix adhesion molecules (Bi *et al.*, 2002). One of these, PH-20, a glycosylphosphatidylinositol-anchored protein with hyaluronidase activity, plays a relevant role in cumulus penetration. PH-20 is initially located on the plasma membrane, and then translocates to the inner acrosomal membrane of acrosome-reacted mammalian sperm (Primakoff *et al.*, 1985; Cherr *et al.*, 1996). PH-20's role in secondary binding is dependent upon repetitive hydrolysis by the hyaluronidase domain of hyaluronic acid present in the ZP (Hunnicutt *et al.*, 1996). Zonadhesin was first detected in a membrane fraction isolated from pig sperm; it showed unique species-specific binding activity, although orthologs have been characterized in mouse and in human (Tardif *et al.*, 2010). ZP3r (also known as sp56) is another adhesion molecule discovered in mouse, but is possibly present in the acrosome of almost all mammalian species. It has been proposed to act in concert with proacrosin in progressive penetration through the extracellular coat (Bleil and Wassarman, 1990), but its involvement in sperm–ZP binding has been recently questioned (Muro *et al.*, 2012).

Studies that addressed how the sperm structures involved in AR participate in the activation process yielded evidence that proteins known to be distributed only in the sperm equatorial segment protein (SPESP) of ejaculated human and hamster sperm are involved; the integrity of these proteins correlates with binding and successive fusion with the oocyte. In further investigations, a mouse line was engineered to lack SPESP1, resulting in aberrant distribution of various sperm proteins. This was related to the fertilizing ability of the sperm and confirmed that SPESP1 is required in order to produce a fully 'fusion competent' sperm (Wolkowicz *et al.*, 2008; Fujihara *et al.*, 2010).

Fusion

The final adhesion between gametes occurs via their plasma membranes, preceding the event of fusion (for review, see Gadella and Evans, 2011). Recent studies identify cell fusion as a genetically programmed process that in gametes can be divided in two main stages: membrane binding and membrane fusion.

Regarding membrane binding, many of the studies that aimed to identify sperm proteins required for sperm–oocyte fusion were based on immunological assays, and suggested several different molecules. The PH-30 protein, also known as fertilin, was localized on the posterior sperm head surface in guinea pig and postulated to be a candidate for mediating gamete membrane fusion (Primakoff *et al.*, 1987). Biochemical characterization of PH-30 showed two subunits, α and β, the latter containing a disintegrin domain, suggesting that an egg integrin might serve as sperm receptor. Due to their ability to inhibit sperm–oocyte binding, integrin ligands for other peptides and cyritestin were also proposed as being involved (Takahashi *et al.*, 2000). A model for sperm–oocyte membrane binding involving adhesion between an integrin on the oocyte plasma membrane and an integrin–ligand on the sperm was also suggested (Talbot *et al.*, 2003); however, subsequent knockout experiments have not supported this view further (Stein *et al.*, 2004, see comment in PubMed Commons below).

Together with cyritestin, fertilin α and β subunits were also characterized as first members of the ADAMs family, metalloproteinases that can bind to receptors and cleave extracellular domains of proteins, for example in protein shedding or activation. These were considered ideal candidates for attachment proteins owing to the presence of specific domains and the fact that they are testis specific. Many proteins belonging to the ADAM family have now been identified, but not all *in vitro* experiments fully support their direct role in fusion (Stein *et al.*, 2004). Accordingly, DE, a rat epididymal protein on the sperm surface, was also suggested as a molecule mediating gamete membrane fusion in the rat and mouse, through a mechanism that does not involve the disintegrin–integrin interaction (Cohen *et al.*, 2000). DE was the first epididymal protein identified as a member of the highly conserved cysteine-rich secretory protein (CRISP) family. Further studies characterized other members of the CRISP family that participate in the regulation of signaling pathways during capacitation and are functional in mammalian sperm–ZP interaction and fusion (Cohen *et al.*, 2011; Da Ros *et al.*, 2015).

CD9, a member of the tetraspanins protein family is expressed on the oocyte surface of many mammalian species; a clear role for CD9 in sperm–oocyte fusion was demonstrated (Miyado *et al.*, 2000), since it participates in the formation of microvilli that are important for sperm–oocyte fusion (Runge *et al.*, 2007). Current findings show that CD9 generates adhesion sites that are responsible for the strongest of the observed gamete interactions (Jégou *et al.*, 2011), confirming an essential role for CD9 in the fertilization process (Jankovičová *et al.*, 2015). However, there is still no evidence for an appropriate sperm ligand partner.

In membrane fusion, morphologically, fusion occurs by lipid mixing that transforms the two gamete membrane bilayers into a single layer, leading to subsequent incorporation of the sperm head into the oocyte cytoplasm. In most cases, initial attachment occurs between the oocyte plasma membrane and the tip of the sperm.

Despite the importance of sperm–oocyte fusion in fertilization, little is known about the mechanisms or the molecules involved. In the mouse, Izumo1 has been identified as essential in sperm–oocyte fusion: a Type I immunoglobulin superfamily membrane protein that is included in the acrosome. After acrosomal exocytosis, fusion between the acrosome and the plasma membrane relocates Izumo1 on the sperm head surface, suggesting that this redistribution renders the sperm fusion competent (Buffone *et al.*, 2014), and therefore plays a pivotal role in the process (Aguilar *et al.*, 2013). Mice deficient in Izumo1 give rise to sperm with normal morphology that can bind and penetrate the ZP, but are incapable of fusing with the oocyte (Inoue *et al.*, 2005). Intracytoplasmic injection of these sperm gives rise to normal fertilization and development, which further corroborates the suggestion that Izumo1 is involved in fusion (Klinovska *et al.*, 2014). It has been hypothesized that Izumo1 is the possible sperm counterpart to CD9, but direct interaction between the two factors has been not reported, and how they might participate in the process has not been shown (Okabe, 2014). Recent findings support the hypothesis that Izumo1 interacts with a protein complex that contains or modulates other fusion molecules (Inoue *et al.*, 2011).

The search for an Izumo1-binding partner in mouse oocytes led to the discovery of Juno, a protein highly expressed on the surface of unfertilized oocytes: incubation with Juno-specific antibody prevents fertilization. Knockout female mice for Juno are infertile, and in particular their oocytes were unable to fuse with wild-type sperm, even in the presence of normal ZP penetration.

Experimental evidence demonstrated rapid loss of Juno from the oocyte membrane soon after fertilization, suggesting that Juno is essential for fertilization and that this mechanism may be the basis for polyspermy block in mammals. One possible explanation for this process is that Juno is shed in vesicles after fertilization, generating a zone of 'decoy oocyte' confined within the perivitelline space that could bind acrosome-reacted sperm and therefore avoid supernumerary sperm entry (Bianchi and Wright, 2014).

Although recent studies have validated Juno protein as the first cell surface receptor conserved in mammals, the interaction between Izumo1 and Juno seems to be a necessary and essential adhesion step, but its role in the gamete fusion mechanism is not clear (Bianchi *et al.*, 2014).

Oocyte activation

Quiescence

At the end of oogenesis, the oocyte has accumulated maternal RNAs and proteins that allow it to remain in a developmentally arrested state. This quiescence is characterized by blocks at both nuclear and cytoplasmic levels. In the majority of species, the oocyte can arrest at different stages of meiotic division, and in particular the block that occurs during first meiotic prophase marks the state of the immature oocyte characterized by the germinal vesicle. In response to a stimulus, meiosis is resumed, manifested by germinal vesicle breakdown and further progression to Metaphase I or II. Depending on the species, meiosis then undergoes a second arrest, which is universally removed by the fertilizing spermatozoon, with the exception of Drosophila (Kaneuchi *et al.*, 2015). Oocytes are also arrested at different points in the cell cycle, mediated by the activity of different types of cytostatic factors (CSF) (Masui, 2001; Costache *et al.*, 2014). The arrest in metaphase of meiosis is characterized by high activity of two types of protein kinases: maturation promoting factor (MPF), whose core components are cyclin-dependent kinase 1 (Cdk1/Cdc2) and cyclin B and CSF, which relies on the MOS/MEK1/MAPK (mitogen-activated protein kinase)/P90^{Rsk} pathway for maintaining the anaphase promoting complex/cyclosome (APC/C) inactive, via the Emi2/Erp1 signaling cascade (Russo *et al.*, 2009). In spite of differences between forms of CSF, the mechanism of CSF inactivation relies on calcium waves that cross the oocyte; in fact, it is known that calcium calmodulin-dependent protein kinase (CAMKII) is the key kinase that transduces the fertilization-induced calcium rise to APC/C-mediated cyclin B destruction and activation of Wee1B; together, these result in the activation of Cdk1 (MPF) and exit from meiosis (Russo *et al.*, 2009; Levasseur *et al.*, 2013).

In mouse oocytes, a CaMKIIγ isoform was shown to be essential for egg activation by triggering cell cycle resumption, which in turn results in independent recruitment of maternal mRNAs by CaMKII activity (Backs *et al.*, 2010).

In the oocyte, precise coordination of these events depends upon the establishment and maintenance of M-phase arrest, as well as prompt release from the arrest points at the time of sperm entry, which fully relies on sperm-induced calcium elevation (Von Stetina and Orr-Weaver, 2011; Hörmanseder *et al.*, 2013).

Cytoplasmic maturity represents an ensemble of parameters that is difficult to fully define, but includes oocyte competence that allows full development of the embryo: initially until the stage of the maternal-to-zygotic transition, and subsequently up to term. Several growth factors and cytokines such as growth hormones, the epidermal growth factor family of proteins, insulin-like growth factors and leukemia inhibiting factor are involved in this process; this can be established in terms of pregnancy and delivery. The immediate increase and mobilization of GSH is achieved through two ATP-dependent steps: gammaglutamylcysteine synthetase and GSH synthetase. The impact of GSH mobilization on further embryonic development is immediate, demonstrated by an increased rate of blastocyst formation and increased cell number per blastocyst (Furnus *et al.*, 2008). This mobilization is mandatory for the sperm head swelling that is necessary for the formation of a fully developed male pronucleus; it is also required for upregulation of the pentose phosphate pathway (PPP), a prerequisite for initiation of the first S-phase in both male and female pronuclei.

Cytoplasmic mRNA polyadenylation of the corresponding mRNAs is mandatory for an immediate translation of all the enzymes involved in GSH synthesis and mobilization, and PPP upregulation. Microarray experiments showed that poly(A)-binding protein is highly expressed in human oocytes (Guzeloglu-Kayisli *et al.*, 2008). The precise selection and regulation of mRNAs to be translated and the quality of their kinetic and chronologic regulation is crucial for correct development. The sequence of message translation is probably regulated by recruitment and degradation of maternal mRNAs and proteins that appear essential to permit the maternal-to-zygotic transition for further development (Sirard, 2011; Lee *et al.*, 2014).

Oocyte activators

The idea that a sperm component may be responsible for oocyte activation and that sperm act through an external receptor dates back to 100 years (Lillie, 1913). More recently, three main hypotheses prevailed, each based on clear experimental evidence; however, contrasting data exist in the literature both in support of and against each of them (for review, see Tosti and Ménézo, 2010).

The calcium conduit model arose from the idea that sperm introduce a quantity of calcium upon fusion, or through its own plasma membrane ion channels. The second model points to a receptor-mediated oocyte activation pathway involving a central role for G-proteins as signal transducers from surface receptors to downstream molecules. Although stimulation of G-protein pathways was supported by many studies, the huge success of the ICSI technique, which bypasses sperm–egg membrane interaction, strongly argues against this theory. At present, this hypothesis has been abandoned: although the presence of a G-protein pathway in the oocyte is not in doubt, it may not necessarily participate in oocyte activation (Williams *et al.*, 1998).

The third hypothesis is based on a diffusible molecule/package of molecules present in the sperm cytoplasm that enters the oocyte cytoplasm after fusion, triggering activation events (Dale *et al.*, 2010). In contrast to the former two theories, a general consensus does support the model of a soluble SF. However, despite many experimental efforts, the real nature of the SF remains to be elucidated. The potential protein nature of the sperm extract was first suggested in 1990 (Stice and Robl, 1990) and subsequently confirmed by other authors, both in mammals and in marine invertebrates (Tosti and Ménézo, 2010). Using biochemical and immunological approaches, the oscillin protein was isolated and cloned from hamster sperm and proposed as the potential candidate (Parrington *et al.*, 1996). Later, it appeared that oscillin was not the SF, and a second era of investigation was initiated at the end of the 1990s (Tesarik, 1998a,b). As a consequence of the central role played by phosphatidylinositol (4,5)-bisphosphate (PIP_2) in mobilization and calcium release at fertilization, PLCs, the enzymes responsible for hydrolysis of the membrane PIP_2, were hypothesized as possibly being involved, suggesting that the SF could be PLC itself. In this respect, the huge search for the correct isoform of PLC soon identified PLCζ as the isozyme that possessed all of the specific characteristics of SF (Swann *et al.*, 2004). In fact, PLCζ satisfies some stringent and independent criteria required for the activating factor in mouse, currently being the only SF candidate able to cause calcium release and target PIP_2 (Kashir *et al.*, 2014). In non-mammalian models, PLCγ isoform and a non-protein package of molecules including ADPribose and nitric oxide (NO) may be involved (Carroll *et al.*, 1999; Dale *et al.*, 2010; Fig. 3).

Recent studies have shown that post-acrosomal WW domain-binding protein (PAWP), a WW-binding domain protein identified on the post-acrosomal sheath of mammalian sperm, exhibits acceptable characteristics for a sperm-borne activating factor (Kashir *et al.*, 2015; Amdani *et al.*, 2015a), suggesting that PAWP may be a promising SF candidate. However, contrasting data demonstrate that PAWP does not play an essential role in mouse fertilization and that it is not able to induce oocyte activation in males who failed to fertilize due to a PLCζ mutation (Escoffier *et al.*, 2015). Furthermore, the exact mechanism by which PAWP induces a calcium rise or oscillations is still unknown (Machaty, 2016). The identity of SF and alternative factors to PLCζ and PAWP are a matter of intense investigation and still need scientific and clinical validation (Satouh *et al.*, 2015; Vadnais and Gerton, 2015; Nomikos *et al.*, 2015).

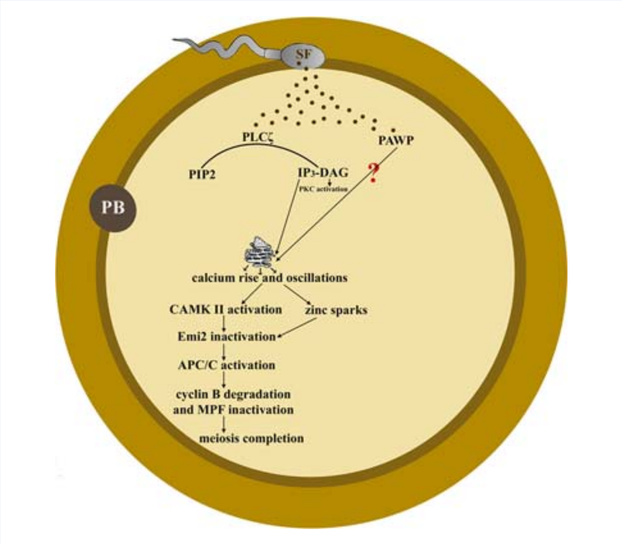


Figure 3 SF pathways leading to activation.
The hypothetical mechanism of action of PLCζ and downstream events in mammalian oocyte activation. Following gamete plasma membrane fusion, the SF diffuses into the ooplasm, binds and hydrolyzes PIP_2 generating two second messengers, namely IP_3 and DAG. IP_3 induces calcium release from the endoplasmic reticulum stores. The calcium rise and oscillations may induce a cascade of translational events, such as CAMKII activation, Emi2 inactivation, APC/C activation, cyclin B degradation and MPF inactivation. Altogether, these events result in the resumption and completion of meiosis. In alternative, calcium rise and oscillations may induce zinc sparks which in turn generate Emi2 inactivation. PAWP is potential SF, which induces calcium rise and oscillations by a still unknown mechanism. DAG, diacylglycerol; SF, sperm factor; PLC, phospholipase C; IP_3, inositol 1,4,5-trisphosphate; CAMKII, calcium calmodulin-dependent protein kinase; APC/C, anaphase promoting complex/cyclosome; MPF, maturation promoting factor; PAWP, postacrosomal WW domain-binding protein; PIP_2, phosphatidylinositol (4,5)-bisphosphate.

Electrical events

The oocyte is electrically excitable, due to ion channels located on the plasma membrane. Changes in the electrical properties of the oocyte plasma membrane are crucial events in the process of oocyte activation (Tosti and Boni, 2004). Pioneering studies on marine animals first demonstrated potassium ion fluxes through the oocyte plasma membrane (Tyler *et al.*, 1956; Hiramoto, 1958), that in turn generated a transient change in oocyte membrane potential, named fertilization potential. The fertilization potential was also recorded in echinoderm oocytes, suggesting that it is generated by the activation of a transient voltage-dependent inward current (Dale *et al.*, 1978; Dale and De Santis, 1981). With the advent of the whole-cell voltage clamp technique, it was demonstrated that depolarization of the membrane potential resulted from ions flowing through the plasma membrane as an ion current, known as the fertilization current (FC). Accurate biophysical characterization in ascidians demonstrated that this FC is due to gating of large non-specific and highly conductive plasma membrane ion channels activated in the oocyte by the fertilizing spermatozoon (Dale and De Felice, 1984; Dale, 1994). FC was recorded in sea urchin and lower vertebrates such as Xenopus, where the channels responsible were characterized as non-specific and calcium-activated chloride channels, respectively (De Simone *et al.*, 1998; Glahn and Nuccitelli, 2003).

The first difference between non-mammalian and mammalian electrical responses was demonstrated in hamster oocytes, where a series of hyperpolarizations following fertilization were recorded in the oocyte (Miyazaki and Igusa, 1981a). A similar FC has subsequently been recorded in other mammalian species, such as mouse (Igusa *et al.*, 1983) and bovine (Tosti *et al.*, 2002). In contrast to other mammals, rabbit oocytes showed a preliminary depolarization followed by repeated diphasic (hyperpolarization/depolarization) membrane modifications (McCulloh *et al.*, 1983).

In the human oocyte, a bell-shaped outward FC accompanied by a long-lasting hyperpolarization of the plasma membrane was recorded (Gianaroli *et al.*, 1994), and a further biophysical characterization of ion channels revealed that in the human FC is underpinned by the activity of calcium-activated potassium channels (Dale *et al.*, 1996), also supporting previous findings in hamster oocytes (Miyazaki and Igusa, 1981b). In contrast to the marine species, where an inward FC first appears and seems to be directly gated by the fertilizing spermatozoon, in some mammalian species, the electrical events follow an initial calcium release that in turn gates calcium-activated potassium channels. Accordingly, Homa and Swann (1994) reported a calcium-activated outward current in human oocytes following cytosolic SF injection, proposed to be a signal of oocyte activation.

Apart from the hypothesized fast block to polyspermy, which is a matter of debate (Dale, 2014), the role of FC in the process of fertilization remains unclear; however, in ascidians a long-term effect of FC on embryo development has been suggested (Tosti *et al.*, 2003).

Calcium release

The elevation of free intracellular calcium is a nearly universal signal that triggers the cascade of events that leads to oocyte activation. After pioneering experiments demonstrating that calcium ionophore induced oocyte activation (Steinhardt *et al.*, 1974), the calcium rise was first described in marine animals as an explosion, suggesting that a single peak was the cause of oocyte activation (Ridgway *et al.*, 1977; Steinhardt *et al.*, 1977). Calcium changes were then observed in all the animals studied (Stricker, 1999) demonstrating that this process is one of the few events of oocyte activation conserved through the course of evolution. The massive increase in cytosolic calcium occurs in different forms, from a single transient peak in non-mammals (Busa and Nuccitelli, 1985; Gillot and Whitaker, 1994) to periodic oscillations in mammalian species, with amplitude and frequency that are crucial for the success of oocyte activation and embryo development (Sun *et al.*, 1994; Ducibella *et al.*, 2002; Miyazaki and Ito, 2006; Whitaker, 2006; Swann and Yu, 2008; Kashir *et al.*, 2013; Krauchunas and Wolfner, 2013). In golden hamster, leading studies associated repetitive hyperpolarization in the oocyte a few seconds after sperm interaction with periodic calcium changes (Miyazaki and Igusa, 1982). This view was further supported in mouse oocytes, where sustained calcium oscillations appeared in activated oocytes, beginning as a transient rise but continuing in a regular pattern lasting from 2 to 30 minutes up to several hours (Cuthbertson and Cobbold, 1985; Miyazaki *et al.*, 1993). The first direct measurements of intracellular calcium changes in human oocytes at fertilization were recorded in the early 1990s (Taylor *et al.*, 1993). These authors fertilized both zona-intact and zona-free donated oocytes, and reported the amplitude, duration and frequency of dramatic intracellular calcium transients, which were very similar to those recorded in mouse. In support of the fact that calcium oscillations are cell-cycle dependent, it was shown that they cease when pronuclei are formed, which is considered to be the end point of oocyte activation (Jones *et al.*, 1995). Subsequent studies reported that calcium oscillations are also essential features of oocyte activation in non-mammals, such as different ascidian species (Russo *et al.*, 1996; Dumollard *et al.*, 2004). Over the last few decades, a large body of literature has focused on dynamic aspects of calcium signaling, discussing how calcium waves and oscillations are transformed into oocyte response. As a general model, the first calcium rise may be followed by an entry of calcium across the plasma membrane through voltage-sensitive calcium channels that respond to membrane depolarization in echinoderms. This entry is followed by a further depolarization, which is involved in the polyspermy block (Runft *et al.*, 2002). In most cases, the influx of calcium is stimulated by the depletion of calcium stores, a mechanism that was termed capacitative or store-operated calcium entry (SOCE) (Putney, 1986). This mechanism involves molecules, such as STIM, ORAI and SERCA, coordinated to refill the calcium stores in order to generate new calcium oscillations. Although the latter is generally accepted as modulating calcium influx, alternative mechanisms such as ion channel activity have been identified in the completion of mouse oocyte activation (Miao *et al.*, 2012; Takahashi *et al.*, 2013; Bernhardt *et al.*, 2015), arguing against SOCE as a unique contributor. The main source of calcium stores is the smooth endoplasmic reticulum, where IP_3 and ryanodine receptors are located (Wakai *et al.*, 2013). IP_3 Type I receptors appear to be fully responsible for calcium oscillations in mammals. The importance of IP_3 in oocyte activation was first highlighted with the demonstration of PIP_2 turnover at fertilization (Turner *et al.*, 1984). The increase of IP_3 at fertilization was later observed in most of the species studied. In recent years, PLCζ has been recognized as the sperm-specific phospholipase that hydrolyzes PIP_2 into IP_3 and DAG, allowing IP_3 binding to receptors and confirming the idea that IP_3 is a second messenger fully involved in calcium-dependent oocyte activation (Stith *et al.*, 1993; Swann and Parrington, 1999; Swann and Yu, 2008). Calcium released from IP_3 receptors in turn diffuses to neighboring receptors, triggering a calcium-induced calcium release (CICR), a regenerative process that a great deal of experimental evidence suggests as the mechanism responsible for generating calcium waves (Whitaker, 2006). In addition to IP_3, other calcium mobilizing agents, the so-called fraternal twin messengers (Lee, 2011), cADPR and nicotinic acid adenine dinucleotide phosphate (NAADP), participate in intracellular calcium store mobilization. Although both cADPR and NAADP remain inadequately studied, the former was indicated as a modulator of ryanodine receptor-mediated CICR, and in sea urchin oocytes a NO-stimulated calcium mobilization pathway involving cADPR was also documented (Willmott *et al.*, 1996). In the same species, the NAADP-induced calcium rise was shown to be mediated by a new class of calcium channels, the two-pore channels (Ramos and Wessel, 2013). These findings shed new light on the coordination between messengers, intracellular stores and ion channels in the formation of the oocyte activation fertilization calcium wave. The manner in which calcium oscillations are decoded and downstream effectors and events are triggered is a subject of intense investigation. It is noteworthy that the mechanisms through which calcium interacts with the cell cycle control machinery rely on APC and the degradation of cyclins. However, the molecular mechanisms whereby the calcium signals induce interruption of meiosis arrest have not been fully clarified.

Structural events

Morphological changes in the oocyte after fusion with the spermatozoon are essential to ensure monospermic fertilization and initiation of embryo development. In most of the species studied, these modifications are related to the action of cortical granules (CG), membrane-bound secretory vesicles unique to mature oocytes that release their contents into the perivitelline space (cortical reaction).

CG distribution and the cortical reaction following calcium oscillation are key factors in successful prevention of polyspermy. Calcium released from the endoplasmic reticulum is dependent on the quality and quantity of mitochondria. These are considered to be major markers of oocyte quality, since a low-mitochondrial DNA copy number results in poor oocyte developmental competence.

In marine animals, the first indication that an oocyte has been successfully fertilized is a rapid modification of its shape. In sea urchins, soon after insemination, a calcium-induced exocytosis of CG gives rise to a dramatic change in the extracellular matrix, leading to elevation of the fertilization membrane which plays the dual role of avoiding supernumerary sperm entry and then protecting the zygote and the embryo (Wong and Wessel, 2004).

In mammals, exocytosis is not a continuous process; in fact, each calcium pulse stimulates a loss of CG (Ducibella *et al.*, 2002), suggesting that in mammals, structural modifications are fully dependent on calcium release. CG exocytose their contents, which in turn cleave the ZP2, mixing with the ZP glycoproteins to give rise to zona hardening (Ducibella *et al.*, 1990). This phenomenon, known as the zona reaction, renders the ZP refractory to further sperm binding and penetration. Ascidian oocytes do not have CG or any comparable organelle, and the oocyte morphological modification consists of a constriction that appears at the animal pole and traverses the oocyte, reaching the opposite pole within 2 minutes. The contraction is preceded by an elevation of calcium that crosses the oocyte as a wave in the same direction as the contraction wave (Brownlee and Dale, 1990). The CGs contain several structural proteins and enzymes that give the fertilization membrane or ZP distinct properties of stability and permeability. A proteomic study in echinoderms identified that the proteins SFE1, SFE9, proteoliaisin and rendezvin are responsible for the construction and assembly of the fertilization membrane. In particular, four major enzymatic activities involved in this event were highlighted: proteolysis, transamidation, hydrogen peroxide synthesis and peroxidase-dependent dityrosine cross-linking (Oulhen *et al.*, 2013).

In mammals, the generation of two second messengers, namely IP_3 and DAG, was suggested as mediating the calcium dependence of the cortical reaction, by inducing calcium release from intracellular stores and the activation of protein kinase C (PKC), respectively, which are responsible for CG exocytosis (Sun, 2003).

In several mammalian species, two classes of proteins, known as v- and t-SNAREs, have been shown to play a role in mediating CG docking and exocytosis. In fact, CG exocytosis was shown to be regulated by a SNARE protein-mediated pathway in both mouse and pig unfertilized oocytes (Liu, 2011; Tsai *et al.*, 2011). Furthermore, an active role for α-SNAP (*N*-ethylmaleimide-sensitive factor attachment protein alpha) and NSF (*N*-ethylmaleimide-sensitive factor) in mouse oocyte CG exocytosis has recently been demonstrated (De Paola *et al.*, 2015).

Ovastacin, a protein of the metalloproteinase family similar to hatching enzymes, is an oocyte-specific astacin that has been cloned and characterized in human and mouse ovaries (Quesada *et al.*, 2004). Ovastacin was recently localized as a component of CG released during the cortical reaction, and identified as responsible for the postfertilization cleavage of ZP2 (Burkart *et al.*, 2012). In order to clarify the molecular basis of gamete recognition, molecular biology assays using ablation of the gene encoding ovastacin and truncated recombinant ZP2 peptides supported the hypothesis that sperm bind to an N-terminal domain on the ZP prior to penetration and fusion. This in turn destroys the sperm-binding domain, corroborating the involvement of ovostacin in the mechanism of polyspermy block and regulation of sperm–oocyte interactions (Avella *et al.*, 2013; Yonezawa, 2014).

Meiosis resumption and molecular changes

The last phase of oocyte activation is resumption and completion of meiosis, leading to polar body extrusion, cleavage of the zygote and embryonic cell divisions. Entry into interphase after meiosis exit occurs only after a large decrease in activity of both MPF and MAPK. In many different species, a decrease in MPF activity follows proteolysis of cyclin B, triggered by proteases of the APC complex which is in turn stimulated by the destruction of Emi2 (Madgwick and Jones, 2007). The interplay between Emi2/Erp1, cyclin B and MPF loss has been considered as the key event of meiotic resumption, in particular promoting the metaphase–anaphase transition and extrusion of the polar body (Suzuki *et al.*, 2010). This is however a matter of debate, especially concerning MAPK inactivation, which in mouse appears to be delayed until the time of pronuclear formation. (Gonzalez-Garcia *et al.*, 2014). In oocytes, the effectors of calcium increase involved in cell cycle restart are PKC, CAMKII, calcineurin and the targets are kinases and phosphatases (Williams, 2002; Backs *et al.*, 2010). Null CAMKII isoform γ mice failed to inactivate CSF and MPF. Based on these and other findings in vertebrates and mammals, this mechanism appears to be related to the key role of CAMKII as transducer of calcium signal stimulation leading to phosphorylation of Emi2/Erp1 and eventually its proteolysis. Such degradation should be followed by Cdk1/cyclin B inactivation and release from the meiotic block (Von Stetina and Orr-Weaver, 2011). This model is proposed as an explanation for the link between calcium release and the loss of MPF activity. The missing link between calcium and decline in MAPK activity remains an open question. However, in some mammalian species, it has been suggested that the delayed decrease in CSF may be related to destruction of Mos and inactivation of MAPK (Duprè *et al.*, 2011), a process that is also supported by studies in ascidian oocytes (Dumollard *et al.*, 2011). Recent attention has focused on a decrease in zinc levels that seems to be involved in activation pathways underlying both oocyte activation and meiosis resumption. In mouse, zinc is released from the oocyte at fertilization (Kim *et al.*, 2011), and this event seems to be induced by calcium signals. Zinc release also appears to be involved in meiosis resumption via an association with modulation of Emi2 activity and Cdk1/Cdc2 phosphorylation (Krauchunas and Wolfner, 2013). In particular, zinc spikes that appear to be related to the oocyte–embryo transition have been detected at fertilization in mammals, establishing a zinc-dependent pathway in meiotic cell cycle regulation of mammalian oocytes (Que *et al.*, 2015; Fig. 3). During the final stages of oocyte activation, there are widespread changes in macromolecules such as proteins and RNAs that are not necessarily involved in meiosis resumption. Changes in proteomes and their composition have been characterized, revealing significant degradation of maternal proteins. This has been attributed to a combination of protein degradation, phosphorylation, post-translational modifications and new translation of maternal RNAs (Krauchunas and Wolfner, 2013). During the final stages of oocyte activation, there are widespread changes in macromolecules such as proteins and RNAs that are not necessarily involved in meiosis resumption. Cytoplasmic polyadenylation involves elongation of the poly(A) tail after export of mRNAs to the cytoplasm. It has been observed and described in the oocytes and early embryos of many animal species, from invertebrates to mammals, and is universally considered to be a regulatory mechanism for protein expression from specific mRNAs. The mediators of this process (cytoplasmic polyadenylation elements and their binding proteins) have been described in detail and some new findings have been reviewed recently (Charlesworth *et al.*, 2013; Martins *et al.*, 2016). The decrease in maternal mRNA in early mouse embryos begins during the final stage of oocyte meiotic maturation (Paynton *et al.*, 1988, Paynton and Bachvarova, 1994). A decrease in translatable maternal RNAs and proteins is then a general feature of mammalian preimplantation development until the time of the maternal-to-zygotic transition (Telford *et al.*, 1990), and even afterwards in some cases. The mechanism for selective degradation of mRNAs in early embryos is unknown; however, it is likely that microRNAs interfering with translation play an interesting role in this regulatory process (Sirard, 2012).

Clinical application for ART

A better understanding of gamete physiology is needed in order to improve the continuing low success rates after ART treatment. Patrizio and Sakkas (2009) calculated that the take-home baby rate per oocyte retrieved never exceeds 10%, even with oocyte donors. In IVF centers, gamete activation is also defined as sperm capacitation and oocyte preparation procedures carried out to aid fertilization and pregnancy rates. *In vitro* sperm preparation is needed for the removal of toxic substances present in the seminal plasma in order to select the best, healthy sperm for ART treatment. To achieve this aim, many methods have been developed for separating sperm from seminal fluid, including swim-up and density gradient centrifugation. Accumulated evidence has demonstrated that diluting the sperm samples with a particular medium improved sperm function and enhanced the competence of sperm (Kim *et al.*, 2015a,b). In this respect, several media have been formulated and substances that stimulate the adenylate cyclase pathway, calcium elevation or have a protective effect against chromatin instability and oxidative stress have been tested to identify the most effective sperm separation method. The introduction of ICSI overcame many of the causes of severe male infertility, especially those due to oligospermic semen. However, it soon became clear that performing ICSI with abnormal sperm can potentially induce paternal adverse effects on embryo development and pregnancy outcome (Janny and Menezo, 1994). At present, artificial sperm activation is offered to improve functionality of testicular sperm extracted from azoospermic patients and to activate sperm from patients with Kartagener's syndrome. Recent studies were aimed at improving sperm motility by adding reagents such as pentoxifylline and theophylline to conventional media (Hattori *et al.*, 2011; Ebner *et al.*, 2011) or by combining sperm processing procedures (Wöber *et al.*, 2015). Encouraging results demonstrated in both cases that pharmacological stimulation of spermatozoa resulted in a significant increase in fertilization rate, blastulation and pregnancy outcome. These authors (Ebner *et al.*, 2011) highlighted the fact that stimulating sperm motility with these substances was not effective *per se*, but allowed an accurate selection of the most viable sperm for ICSI. The key role of ion channels in sperm motility and male fertility is at present under intense investigation. Since CatSper genes seem to have evolved exclusively for sperm function, and only CatSper and Ksper are involved in male fertility disorders, these ion channels appear to be ideal tools for both contraception and for male infertility treatment for ART (Singh and Rajender, 2015). The rare cases of failed fertilization after ICSI are mainly caused by a lack of oocyte activation. Due to the prominent role of an increase in calcium during this process, it was soon clear that the use of calcium ionophores, such as ionomycin and A23187, may be efficiently used in couples who experienced low fertilization rate or even complete fertilization failure after ICSI (Tesarik and Sousa, 1995). Many studies reported that artificial oocyte activation improved fertilization and pregnancy rates in patients with histories of poor fertilization, and even no fertilization, in previous ICSI cycles (Montag *et al.*, 2012; Yeste *et al.*, 2015). A number of research groups also reported cases of successful pregnancies in couples where ionophore was used to activate oocytes and ICSI was performed with oligoasthenoteratospermic semen (Sugaya, 2010; Isachenko *et al.*, 2010), small acrosome or globozoospermic sperm (Heindryckx *et al.*, 2005; Taylor *et al.*, 2010) and also on *in vitro* matured oocytes due to polycystic ovary syndrome in the women (Kim *et al.*, 2015a,b). Furthermore, this procedure was also used in cases of unexplained female infertility or diminished ovarian reserve (Check *et al.*, 2010). Ionophore oocyte activation has recently also been applied as an option in a case of theophylline-resistant Kartagener syndrome patients, leading to a healthy twin birth. This promising result supports the possible routine application of ionophore in patients with primary ciliary dyskinesis (Ebner *et al.*, 2015). Several studies reported that chemical oocyte activation performed by treatment with strontium chloride resulted in successful pregnancies after the use of either ejaculated (Chen *et al.*, 2010) or frozen-thawed testicular sperm (Kim *et al.*, 2012). Although the combination of ICSI and chemical oocyte activation resulted in pregnancies and the birth of healthy babies, in some cases where sperm were unable to fertilize oocytes even after their successful introduction into the oocytes via ICSI, electrical stimulation of the oocytes resolved the problem (Yanagida *et al.*, 1999). This physical stimulus promotes the formation of pores in the plasma membrane and increases the calcium permeability with consequent elevation of calcium concentration. In a randomized study, electrical pulses were applied to oocytes that failed to fertilize after ICSI, showing a significant resumption of embryonic developmental events after electrical activation of the oocytes (Manipalviratn *et al.*, 2006). Finally, the mechanical stimulus of aspirating cytoplasm during ICSI was also shown to lead to high fertilization rates (Ebner *et al.*, 2004). In some cases, failed fertilization post-ICSI has also been associated with sperm protamine deficiency and premature chromosomal condensation (PCC). Artificial activation of such oocytes resulted in an increased fertilization rate, with the exclusion of cases where sperm presented PCC induced by protamine defects (Nasr-Esfahani *et al.*, 2007). Since PLCζ has been recognized to play a role as an oocyte activator, certain types of male infertility have been associated with its absence or reduced levels, as well as defective forms and mutations (Ramadan *et al.*, 2012). Impairment of activating factors causes delayed or abnormal oocyte activation, and this can lead either to fertilization failure or to various anomalies in embryonic development (Tesarik, 1998a,b). In particular, delay in sperm pronucleus formation causes a delay in the process of sperm DNA demethylation, altering DNA repair capacity. Active demethylation and immediate remethylation in the male pronucleus is perturbed, along with dynamic reprogramming of DNA methylation. This leads to a delay in zygote formation, with developmental perturbations including abnormal timing and disruption of the imprinting/DNA methylation maintenance by the DNA methyltransferases I (Market Velker *et al.*, 2012).

The aim of artificial oocyte activation is to mimic physiological mechanisms, based mainly on calcium changes; recent evidence suggests that human recombinant PLCζ may be a novel therapeutic agent for injection into the oocyte in order to rescue activation deficiency, since it promotes calcium oscillations in a dose-dependent way (Yoon *et al.*, 2012; Ramadan *et al.*, 2012; Sanusi *et al.*, 2015; Chithiwala *et al.*, 2015). In contrast to other PLC isoforms, PLCζ can potently stimulate calcium oscillations in oocytes, even if it is apparently not bound to plasma membrane PIP_2. However, PLCζ seems to interact with intracellular vesicles containing PIP_2. Thus, the application of recombinant human PLCζ protein in a series of diagnostic and therapeutic protocols is currently an interesting strategy for subfertile male patients deficient in PLCζ who are undergoing ART (Nomikos, 2015; Amdani *et al.*, 2015b). At present, artificial oocyte activation is successfully applied in many IVF centers all over the world; however, the efficacy and safety of this treatment are not yet established. Conflicting data report either risks associated with manipulating the initial stages of development, and/or reassuring healthy live births (for review, see Vanden Meerschaut *et al.*, 2014). The greatest concern arising from the use of artificial activators is potential interference with the physiological mechanisms of oocyte activation, with respect to the spatially and temporally uncontrolled action of calcium increase, its effect on cell homeostasis and on the downstream cascade of events (Santella and Dale, 2015). These concerns, together with possible epigenetic effects that may be transmitted to the offspring, argue against routine clinical application of such manipulations for treating human infertility; their use should instead be limited to cases of unexplained infertility or recurrent failed fertilization after ICSI. In this respect, in 2012, the Scientific and Clinical Advances Advisory Committee (www.hfea.gov.uk/docs/2012-06-20_SCAAC) was asked to provide guidance in order to authorize licensed centers for the application of these novel processes. It was suggested that centers must perform artificial oocyte activation only in selected patients, such as those with PLCζ deficiency, and that the rationale for using calcium ionophore for individual cases should be documented, ensuring that patients are fully informed about the efficacy and potential risks (http://www.hfea.gov.uk/139.html). Another emerging clinical frontier for achieving genetically related children in couples for whom gamete donation is their only option, is the use of artificial gametes, that is, gametes generated by manipulation of their progenitors or somatic cells. At present, fertilization of a human artificial oocyte after haploidization has been performed although no study has yet reported the birth of human offspring from artificial gametes. Validation of the safety and efficiency of human artificial gametes is still preliminary, and potential clinical application of these studies is a challenge for the scientific community that requires extensive basic and clinical research as well as serious socio-ethical and legal considerations (for review, see Hendriks *et al.*, 2015a,b).

Future perspectives

In spite of the fact that gamete activation plays a key role in fertilization, many molecular mechanisms that accompany this process remain to be elucidated. For decades, calcium release and downstream events have been recognized as playing an essential role in sperm–oocyte interaction; the evidence that sperm PLCζ causes calcium oscillations in the oocytes of several different mammalian species attributes the properties of sperm activating factor to PLCζ (Swann and Lai, 2016). On this basis, artificial oocyte activation may be useful in rescuing severe male infertility and associated developmental problems (Ebner and Montag, 2016). However, these advanced techniques may not be routinely available in the majority of IVF centers. At present, it is clear that the chance for successful fertilization strongly depends on fully competent gametes. Therefore, one of the major challenges in IVF lies in obtaining new accurate diagnostic tools for sperm and oocyte quality. The quantification and localization of PLC isoforms within sperm may represent new diagnostic biomarkers for sperm fertilization potential (Ramadan *et al.*, 2012), but it is more difficult to identify the mechanisms involved in the acquisition of oocyte competence. Numerous molecular methods targeted toward evaluating and selecting the ideal oocyte have recently been developed. At present, mRNA profiles based on genomic/transcriptome analysis appear to be a challenging approach toward understanding oocyte quality. However, since extracting mRNA without destroying the oocyte is not feasible, identifying proxies among follicular cells such as cumulus and granulosa cells seems to be an alternative approach for identifying biomarkers of oocyte quality, overcoming the limitations related to individual variation of mRNA composition in different oocytes (reviewed by Labrecque and Sirard, 2014). The post-transcriptional step is still a 'gray box', although microRNAs have opened a new and interesting strategy. One often neglected point is the basic metabolism of the oocyte and its deregulation with oxidative stress and methylation errors, commonly aggravated by endocrine disruptors. Basic metabolism is rarely taken into account, but both problems have common denominators: both affect the tertiary structure of the nucleus. Proteomics and metabolomics, that is, protein profiling of the compounds released, should lead to new advances. Intermediate metabolism should be focused on the 1-C cycle in cumulus-enclosed and naked oocytes: GSH is a key metabolite in oocyte activation, and its counterpart, homocysteine is a key protagonist in this pathway, with folic acid at the center of heavy trafficking of these molecules in the oocyte. There is a real need for noninvasive biomarkers, but this is not an easy task: the question of feasibility is still unknown with current technologies, due to the minute quantity of material available (Nel-Themaat and Nagy, 2011; McRae *et al.*, 2013). Moving forward, parthenogenetic activation of the oocyte is of great interest, with the scope of creating human embryonic stem cell lines for use in cell and tissue therapies. Promising findings in this field have already demonstrated the potential use of stem cell lines in regenerative medicine, circumventing ethical and legal problems arising from the use of human embryonic cell lines. Nonetheless, caution must be exercised in order to ensure the safety of using cell lines for producing a range of cell types, and particularly in the case of cell and tissue transplantation (Bos-Mikich *et al.*, 2016). Identifying biomarkers of gamete quality is crucial in increasing the potential contribution of biotechnologies related to reproduction and ART technology.

Concluding remarks

In this review, we have summarized results surrounding research into the cascade of events associated with the transition from arrested to developmentally competent cells, the process known as gamete activation. Although we have described these events in a stepwise manner, it is clear that they are intimately connected, sharing most of the molecules and the signaling pathways involved. The majority of the evidence suggests that calcium represents the key molecule involved in each step of mutual gamete activation in all of the species studied.

We have reported the general principles that emerge from comparative studies, taking into consideration the canonical animal models used for experimental research. In particular, we have focused on sea urchin and ascidians as non-mammalian models, the two marine species that for more than a century have made a substantial contribution to knowledge about mechanisms of fertilization (Monroy, 1986; Satoh, 1994), providing the basis for research in mammalian species, including human. Before the advent of human IVF, the mouse model dominated studies on mammalian reproduction. Nowadays, human material is provided from IVF centers, but research on the mechanisms of human reproduction remains difficult, due to the scarcity of material, sub-optimal quality and ethical concerns. As mentioned in the previous section, new clinical challenges aim to manipulate gamete activation in order to improve the fertilization rate in IVF; however, a new view of research is emerging, that is, elucidating the dynamics that govern the passage from the quiescent to pluripotent cell may help in understanding the regulation of stem cells. Although the use of stem cell technology is in the initial stages so far, the potential clinical applications of ovarian-derived stem cells, *in vitro* derived sperm and oocytes from pluripotent stem cells represent a promising alternative resource for treating infertility (Duggal *et al.*, 2014; Volarevic *et al.*, 2014; Moreno *et al.*, 2015). A great deal still remains to be understood about the role of cellular and extracellular environments, cell adhesion molecules and stimuli responsible for either reinforcing the quiescent state, or leading to activation into cell cycle progression.

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Authors' role

ET designed the study, conducted the literature search, drafted and revised the manuscript. YM drafted and revised the manuscript.

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Conflict of interest

Authors declare no conflict of interest.

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