REPRODUCTION
REVIEW

Single Ca^{2+} transients vs oscillatory Ca^{2+} signaling for assisted oocyte activation: limitations and benefits

Minerva Ferrer-Buitrago[1], Davina Bonte[1], Petra De Sutter[1], Luc Leybaert[2,*] and Björn Heindryckx[1,*]

[1]*Ghent-Fertility and Stem Cell Team (G-FaST), Department for Reproductive Medicine, Ghent University Hospital, Ghent, Belgium and* [2]*Physiology Group, Department of Basic Medical Sciences, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium*

Correspondence should be addressed to B Heindryckx; Email: Bjorn.Heindryckx@UGent.be

*(L Leybaert and B Heindryckx contributed equally to this work)

Abstract

Oocyte activation is a calcium (Ca^{2+})-dependent process that has been investigated in depth, in particular, regarding its impact on assisted reproduction technology (ART). Following a standard model of signal transduction, Ca^{2+} drives the meiotic progression upon fertilization in all species studied to date. However, Ca^{2+} changes during oocyte activation are species specific, and they can be classified in two modalities based on the pattern defined by the Ca^{2+} signature: a single Ca^{2+} transient (e.g. amphibians) or repetitive Ca^{2+} transients called Ca^{2+} oscillations (e.g. mammals). Interestingly, assisted oocyte activation (AOA) methods have highlighted the ability of mammalian oocytes to respond to single Ca^{2+} transients with normal embryonic development. In this regard, there is evidence supporting that cellular events during the process of oocyte activation are initiated by different number of Ca^{2+} oscillations. Moreover, it was proposed that oocyte activation and subsequent embryonic development are dependent on the total summation of the Ca^{2+} peaks, rather than to a specific frequency pattern of Ca^{2+} oscillations. The present review aims to demonstrate the complexity of mammalian oocyte activation by describing the series of Ca^{2+}-linked physiological events involved in mediating the egg-to-embryo transition. Furthermore, mechanisms of AOA and the limitations and benefits associated with the application of different activation agents are discussed.

Reproduction (2017) **155** R105–R119

Introduction: Signal transduction during oocyte activation: role of Ca^{2+}

Calcium (Ca^{2+}) is an essential component in orchestrating cell signaling. Calcium levels are finely regulated to stimulate diverse cellular programs while avoiding cellular toxicity or death induced by prolonged and sustained Ca^{2+} changes. Calcium also has a particular duality during signal transduction, acting either as the stimulus or the product during the same process (Miyazaki 1993, Bootman *et al*. 2002). Contributing to this versatility, Ca^{2+} acts as a second messenger to convey diverse stimuli into distinct patterns of Ca^{2+} fluctuations, which can be manifested as transient rises, sustained responses or repetitive oscillatory changes, thereby transducing the specific message into a unique signal (Berridge *et al*. 2000, Bootman *et al*. 2001). Oocyte activation is a well-known Ca^{2+}-dependent process in which the Ca^{2+} signal can be relayed as a single Ca^{2+} transient (i.e. a Ca^{2+} elevation followed by recovery), further called a 'single' response or can attain a more dynamic mode of repetitive Ca^{2+} transients called 'Ca^{2+} oscillations'. A single Ca^{2+} transient is characterized by an amplitude and duration, whereas Ca^{2+} oscillations are additionally characterized by the frequency of the Ca^{2+} events. Altogether, the occurrence of single Ca^{2+} transients vs dynamic Ca^{2+} oscillations depend on the species, with different species displaying specific Ca^{2+} signatures (Stricker 1999). The molecular basis underlying these differences within the animal kingdom has attracted significant interest.

The hallmarks for oocyte activation among numerous species were compared by (Stricker 1999). The specific Ca^{2+} patterns supporting oocyte activation are not necessarily associated with the lineage origin, since species from the same phyla may manifest different Ca^{2+} patterns, all allowing meiotic resumption. For instance, frogs (sp. *Xenopus laevis*) display single Ca^{2+} changes while mice (sp. *Mus musculus*) manifest an oscillatory Ca^{2+} response. Both species have been widely used as models to investigate the cellular mechanistic associated with distinct Ca^{2+} signatures and their impact on oocyte activation. Despite the Ca^{2+} profile differences between both species, the process of oocyte activation is similar (Perry & Verlhac 2008). Briefly, upon fertilization,

ISSN 1470–1626 (paper) 1741–7899 (online)
https://doi.org/10.1530/REP-17-0098
Online version via www.reproduction-online.org

the sperm releases the soluble factor phospholipase C zeta (PLCζ) into the ooplasm. PLCζ hydrolyzes phosphatidylinositol bisphosphate (PIP2), located in oocyte vesicle membranes, into inositol trisphosphate (IP3), which diffuses in the cytoplasm, and diacylglycerol (DAG) which remains associated with the membrane and activates the protein kinase C (PKC) (Gonzalez-Garcia *et al*. 2013). IP3 binds to its cognate receptor (IP3R) embedded on the endoplasmic reticulum (ER), resulting in the opening of Ca^{2+}-permeable channels that facilitate Ca^{2+} release from intracellular stores, thereby increase concentrations of free cytoplasmic Ca^{2+} (Miyazaki *et al*. 1992). Subsequently, a signaling cascade is activated to alleviate meiotic arrest, which is secured by high levels of M-phase promoting factor (MPF) (Tripathi *et al*. 2010). Interestingly, species that physiologically undergo Ca^{2+} oscillations during oocyte activation are also capable of responding to a single Ca^{2+} transient, for instance, as induced by Ca^{2+} ionophores or ethanol during assisted oocyte activation (AOA). Although the efficiency of these artificial methods is established, it is unsure whether a few Ca^{2+} transients induced over a period of a few minutes can recapitulate the signaling effects of the long-lasting Ca^{2+} oscillatory signature. In this regard, the importance of the spatiotemporal aspects of Ca^{2+} oscillations during oocyte activation and its impact on embryo development is still under discussion. This review starts with a description of the physiology of oocyte activation in species which manifest either a single Ca^{2+} transient or an oscillatory Ca^{2+} regime. We then discuss how agents such as Ca^{2+} ionophores alleviate meiotic arrest by inducing a single or a few Ca^{2+} transients in mammalian oocytes. Furthermore, we discuss the limitations and benefits of diverse AOA protocols and how they have an impact on both early and late events of embryo development.

Oocyte activation: Physiology of Ca^{2+} transients and oscillations

For clarity, the discussion that follows will be organized in four parts, each linked to a fundamental aspect of the Ca^{2+} signaling cascade (Fig. 1), as suggested by Berridge and coworkers (Berridge *et al*. 2000). The model encompasses the stimulus, the ON mechanisms, the effectors and the OFF mechanisms. These four steps allow to structure the numerous findings related to oocyte activation, further outlining which conditions promote single Ca^{2+} transients or Ca^{+2} oscillations in different species.

The stimulus: PLCζ

It has been well documented that oocyte activation initiates when sperm delivers a specific soluble factor to the ooplasm upon gamete fusion in all mammalian species studied to date (reviewed by Kashir *et al*. 2013, Swann & Lai 2013). Phospholipase C ζ was identified as the sperm-borne soluble factor that triggers oocyte activation (Cox *et al*. 2002, Saunders *et al*. 2002). In the case of non-mammalian vertebrates, the model of the 'soluble factor' has been demonstrated only in newt

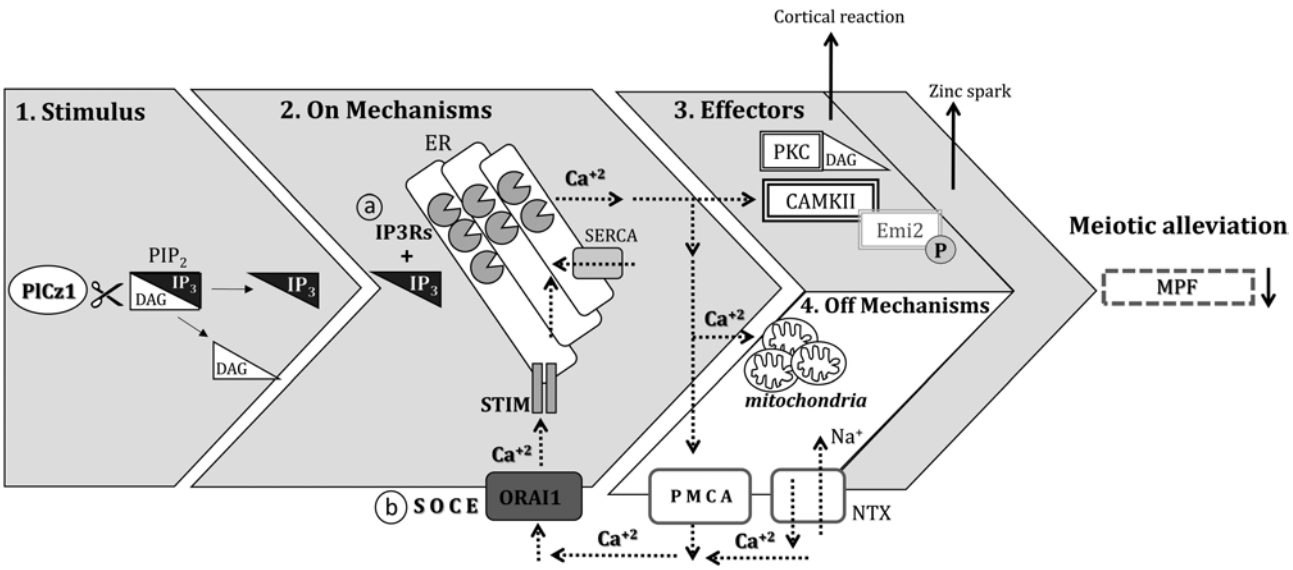

Figure 1 Schematic diagram of fundamental aspects of the Ca^{2+} response during oocyte activation based on the model proposed by Berridge *et al*. (2000). Broken lines represent Ca^{2+} mobilization throughout the process. (1) Stimulus. PLCζ mediates IP3 and DAG production by PIP2 hydrolysis. (2) On mechanisms. (2.a) IP3R opens in response to IP3 allowing an increase of free cytoplasmic Ca^{2+} with Ca^{2+} depletion from endoplasmic reticulum (ER). (2.b) SOCE facilities Ca^{2+} influx in response to ER Ca^{2+} depletion. (3) Effectors. Ca^{2+} trigger initiates a signaling cascade, which activates effectors such as CAMKII. (4) Off mechanisms. Excess of cytoplasmic Ca^{2+} is restored to baseline levels by (4.a) SERCA pumps, (4.b) NCX, (4.c) PMCAs and (4.d) mitochondria.

(sp. *Cynops pyrrhogaster*) (Harada *et al*. 2011). *Xenopus* fertilization is triggered by a single Ca^{2+} transient (Nuccitelli *et al*. 1993) and although 'PLCζ counterpart' has not yet been identified, a gene identity of 42% to the mouse PLCζ protein has been reported (Runft *et al*. 1999, 2002). PLCζ triggers Ca^{2+} oscillations in mammals (Cox *et al*. 2002). It is a soluble and Ca^{2+}-sensitive enzyme that catalyzes the hydrolysis of PIP_2 at basal concentrations of Ca^{2+} to generate IP_3 and DAG (Miyazaki 1993). The signaling cascade of DAG proceeds, in concert with Ca^{2+}, toward activation of PKC (Ducibella & Fissore 2008, Halet 2004). PKC signaling, however, does not initiate meiotic release in mouse oocytes (Madgwick *et al*. 2005). PIP_2 was recently proposed to be distributed in intracellular vesicles in mouse oocytes, rather than being clustered at the plasma membrane as is the case in somatic cells (Halet *et al*. 2002). PLCζ has the specific capacity to bind directly to these internal PIP_2-containing vesicular membranes (Yu *et al*. 2012) that are hypothetically formed from Golgi and other membrane-trafficking systems upon metaphase II arrest (Payne & Schatten 2003). The clustered distribution of the PIP_2-containing vesicles (Yu *et al*. 2008) allows the propagation of the Ca^{2+} wave upon sperm–oocyte fusion in response to the release of PLCζ into the oocyte (Deguchi *et al*. 2000).

The frequency of Ca^{2+} oscillations depends in part on the IP3 concentration (Mehlmann *et al*. 1996, Malcuit *et al*. 2005); hence, it is not unexpected that the oscillation frequency is also dependent on PLCζ concentration. As a result, PLCζ concentrations are correlated with sperm activation potential (Heytens *et al*. 2009, Nomikos *et al*. 2014*b*). It is worth highlighting that the PLCζ mechanism does not show specificity among vertebrates (Cox *et al*. 2002, Coward *et al*. 2011), therefore, heterologous oocyte activation has been used as a strategy to discern the role of PLCζ' in modulating Ca^{2+} dynamics among different species (reviewed by Ito *et al*. 2011). Interestingly, *Xenopus* extracts failed to initiate oocyte activation after microinjection into conspecific oocytes (Harada *et al*. 2011) and were able to induce a Ca^{2+} oscillatory response in *Cynops* (Harada *et al*. 2011) and mouse oocytes (Dong *et al*. 2000). Although the Ca^{2+} oscillation properties are in part determined by the PLCζ concentration, they are additionally influenced by the properties of the oocyte Ca^{2+} signaling machinery, which is discussed next.

ON mechanisms

The ON mechanisms mediate a cytoplasmic Ca^{2+} increase required for oocyte activation (Kline *et al*. 1999). Oocyte acquires its ability for Ca^{2+} release during fertilization during final oocyte maturation (Ajduk *et al*. 2008), with different Ca^{2+} channels controlling the Ca^{2+} release from internal stores, such as type 2 and 3 ryanodine receptors (Ayabe *et al*. 1995) and type 1 inositol 1,4,5-trisphosphate receptor (IP3R1), or the entry of external Ca^{2+}, for instance via store-operated calcium entry (SOCE) (Machaty 2016).

Inositol trisphosphate receptors (IP3Rs)

Mouse studies have shown that, although ryanodine receptors are present and may potentiate IP3-triggered changes in Ca^{2+} concentration, their contribution is not critical in inducing oocyte activation (Ayabe *et al*. 1995). As a result, IP3-induced Ca^{2+} release (IICR) is the predominant mechanism. Experiments in *Xenopus* suggest that the channel of IP3R1 opens through a conformational transition in response to IP3 and Ca^{2+} concentrations (Shuai *et al*. 2009).There are three IP3R isoforms described (types 1, 2 and 3) in mice (Fissore *et al*. 1999), of which IP3R isoform 1 is particularly expressed (Parrington *et al*. 1998). The IP3R isoform 1 is also predominant in frogs (Kume *et al*. 1993). Immature oocytes at their germinal vesicle (GV) phase are less sensitive to the IP3-induced Ca^{2+} release (IICR) than oocytes at the metaphase II (MII) stage (Mehlmann & Kline 1994). This dramatic increase in sensitivity to IP3 arises from variations such as an increase of IP3R levels, IP3R redistribution (Mehlmann *et al*. 1996, Goud *et al*. 1999) and post-translational modifications (Vanderheyden *et al*. 2009*a*,*b*), in particular phosphorylation. M-phase kinases, such as polo-like kinase 1 (Plk1) (Ito *et al*. 2008), mitogen-activated protein kinase (MAPK) (Lee *et al*. 2006) and cyclin b/cyclin-dependent kinase 1 (Cdk1) (Zhang *et al*. 2015), play an important role in modulating this increase in the sensitivity of the IP3R. Polo-like kinase 1 participates in the early phases of oocyte maturation (Ito *et al*. 2008). Mouse studies using a Plk1 inhibitor demonstrated that Plk1 phosphorylates an MPM-2 epitope on the IP3R1 (Vanderheyden *et al*. 2009*a*,*b*). Also in *Xenopus*, Plk1 affects the phosphorylation status of the IP3R, but in contrast, there is no evidence supporting its role in sensitizing IP3R. MAPK mediates the phosphorylation of IP3R at MPM-2 during maturation in both *Xenopus* (Sun *et al*. 2009) and mouse oocytes (Lee *et al*. 2006, Vanderheyden *et al*. 2009*b*), leading in turn to their sensitization. However, the phosphorylation site appears to differ between the species (Lee *et al*. 2006, Sun *et al*. 2009). Moreover, Cdk1, a subunit of MPF, targets the IP3R to enhance its sensitivity during mouse oocyte maturation (Wakai *et al*. 2013). Interestingly, *Xenopus* data showed that both Cdk1 and MAPK require simultaneous activation to mediate full sensitization of the IP3Rs (Sun *et al*. 2009). Kinases other than Plk1, MAPK and Cdk1 have also been implicated in IP3R-mediated Ca^{2+} release (Bezprozvanny 2005, Vanderheyden *et al*. 2009*a*,*b*), including PKA, PKC and in particular, Ca^{2+}/calmodulin-dependent kinase II (CaMKII) (Ducibella & Fissore 2008), a key effector during oocyte activation.

Moreover, the ability of IP3R to conduct Ca^{2+} has been associated with an increase in the concentration of Ca^{2+} in the ER ($[Ca^{2+}]_{ER}$) (Cheon *et al*. 2013), which results from the regulation of mechanisms controlling cytoplasmic Ca^{2+} influx and efflux.

Store-operated calcium entry (SOCE)

ER Ca^{2+} content increases during oocyte maturation (Cheon *et al*. 2013) and throughout oocyte activation to maintain a Ca^{2+} oscillatory regime (Wakai *et al*. 2013). In this regard, store-operated Ca^{2+} entry underlies one of the mechanisms facilitating Ca^{2+} influx in response to $[Ca^{2+}]_{ER}$ depletion. In mammals, the two major SOCE players are the stromal interaction molecule (STIM) 1 and 2 (Cahalan 2009), and the Ca^{2+}-release activated Ca^{2+} channel protein 1 (Orai1). STIM 1 and 2 are located in the ER membrane, and Orai1 in the oocyte plasma membrane. STIM2 is activated by limited depletions of Ca^{2+}, while STIM1 is activated when the ER is drastically depleted in Ca^{2+} content. STIM1 functions as a Ca^{2+} sensor that monitors $[Ca^{2+}]_{ER}$ by its EF hand domain (Lewis 2007, Carrasco & Meyer 2011). When the $[Ca^{2+}]_{ER}$ experiences a drastic decrease, STIM1 responds by clustering into large puncta (Stathopulos *et al*. 2006, Liou *et al*. 2007) and migrating toward the ER cortex. STIM1 clusters at the cortex interact with, and open, the Orai1 Ca^{2+} channel, allowing Ca^{2+} entry into the oocyte (Cahalan 2009, Hogan *et al*. 2010). A recent study demonstrates that *Xenopus* oocytes, expressing an activated form of SOCE only at metaphase II (MII) stage (Machaca & Haun 2000), can adjust Ca^{2+} signaling to single transients or dynamic oscillations depending also on $[Ca^{2+}]_{ER}$, with a drastic Ca^{2+} depletion favoring a single response vs mild Ca^{2+} depletions favoring Ca^{2+} oscillations (Courjaret *et al*. 2017). SOCE contributes to the increase in intracytoplasmic Ca^{2+} and its function modulates Ca^{2+} oscillations in certain species as swine (Lee *et al*. 2012). However, several findings suggest that SOCE might not significantly contribute in mediating Ca^{2+} influx to maintain long lasting Ca^{2+} oscillations in for example mouse (Takahashi *et al*. 2013). Mouse oocytes cultured in Ca^{2+}-free medium failed to get activated after sperm microinjection while inhibition of SOCE in the presence of extracellular Ca^{2+} was associated with normal activation (Miao *et al*. 2012). These observations indicate that Ca^{2+} influx is essential for oocyte activation and suggest the participation of Ca^{2+} entry pathways other than SOCE during oocyte activation, at least in mouse.

Effectors: CAMKII

CaMKII is considered as the major effector during oocyte activation in mammalian vertebrates (Lorca *et al*. 1993, Backs *et al*. 2010). This kinase selectively phosphorylates upstream targets like IP3R, resulting in its inhibition, and downstream targets leading to the degradation of the early mitotic inhibitor 2 (Emi2), for instance (Perry & Verlhac 2008). CaMKII is a multifunctional protein sensitive to the Ca^{2+}–calmodulin complex, and it is typically activated in a frequency-dependent manner by Ca^{2+} oscillations as demonstrated in mammals (Tatone *et al*. 2002). CaMKII can also be activated by high cytoplasmic concentrations of Ca^{2+}, through a cascade involving autophosphorylation and achievement of a sustained active status (Markoulaki *et al*. 2003, Dupont *et al*. 2010). In general, CAMKII shows lower activity in oocytes than in other cell types (Markoulaki *et al*. 2003); its activity depends on the Ca^{2+} oscillations frequency as well as the *duty cycle*, i.e. the percentage of time that the Ca^{2+} signal is high, relative to the duration of the oscillatory period (Smedler & Uhlén 2014). As a result, CaMKII activity depends on the integration of the preceding Ca^{2+} dynamics, i.e. the time during which the Ca^{2+} signal is high will determine the CaMKII activation status in which the frequency as well as the duty cycle play a role. Thus, low frequencies and short spike durations will trigger little CAMKII activity while continued spiking at higher frequencies will activate and maintain CAMKII activity. The influence of the duration of the Ca^{2+} transients during fertilization has been illustrated in several studies. The first Ca^{2+} transient upon sperm entry always shows a longer duration than subsequent oscillatory spikes (reviewed by Halet 2004, Miao & Williams 2012). In particular, in human oocytes, the duration of the first transient is in the order of 3–4.5 min, whereas subsequent transients have a shorter duration (2.2–2.8 min) (Nikiforaki *et al*. 2014*b*). Interestingly, when activation is artificially induced by ionomycin at a low concentration of 2 µM, Ca^{2+} returns nearly to its basal level from its peak value within 5 min, when CAMKII registers 64% of its maximal activity at the occasion of the very first Ca^{2+} transient (Markoulaki *et al*. 2003). Moreover, long-duration Ca^{2+} transients are not invariably effective; in fact, one study in mouse oocytes has demonstrated that phosphatases can mediate CAMKII inactivation in the presence of sustained Ca^{2+} levels (Ozil *et al*. 2005). Taken together, these findings suggest that the duration of both isolated Ca^{2+} transients, as well as ongoing oscillatory Ca^{2+} dynamics, contribute to activate and to maintain CAMKII active throughout meiosis completion.

OFF mechanisms

In order to sustain an ongoing Ca^{2+} oscillatory activity over several hours, it is necessary to have a set of OFF mechanisms that ensure restoration of the Ca^{2+} levels to baseline after each spike (Brandman *et al*. 2007, Cahalan 2009). In general, OFF mechanisms include refilling of the ER Ca^{2+} stores by sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPases (SERCA pumps), extrusion of Ca^{2+} by low-affinity, high-capacity Na^{+}/Ca^{2+} exchange

proteins (NCX) and high-affinity, low-capacity Ca^{2+} ATPases in the plasma membrane, as well as and Ca^{2+} uptake into mitochondria.

PMCAs and SERCA pumps

Presence of NCX has been reported in mouse oocytes (Carroll 2000). However, Ca^{2+} efflux mediated by PMCAs is suggested to be more relevant. Studies in *Xenopus* show that PMCAs experience an internalization during oocyte maturation leading to a sustained single Ca^{2+} rise mediate fertilization (El-Jouni *et al*. 2008). The impact of PMCA internalization on modulating Ca^{2+} changes is observed as follows. While an immature oocyte manifests a sharp and fast Ca^{2+} spike upon sperm microinjection, a mature oocyte displays a Ca^{2+} rise that lasts for at least 5 min (El-Jouni *et al*. 2008). These observations suggest the contribution of PMCA to Ca^{2+} efflux. Additionally, intracytoplasmic Ca^{2+} is taken up into ER lumen by SERCA pumps (Wakai *et al*. 2012), and it occurs after Ca^{2+} influx. Both events, thus Ca^{2+} influx and ER replenishment, play an important role in guaranteeing the persistence of Ca^{2+} oscillations during mammalian oocyte activation.

Mitochondria

Mitochondria are also actively involved in Ca^{2+} homeostasis during fertilization (Rizzuto *et al*. 1998). Mitochondrial ATP production is necessary for SERCA activity (Fissore *et al*. 2002), a mechanism involved in ER replenishment (Wakai *et al*. 2013). Mitochondria are associated with the maintenance of low basal cytoplasmic Ca^{2+} levels contributing to the occurrence of the Ca^{2+} oscillations (Dumollard *et al*. 2004). In addition, mitochondrial Ca^{2+} uptake has also been shown to be essential in the regulation of IP3-induced Ca^{2+} release (IICR) during Ca^{2+} signaling in both, *Xenopus* and mice oocytes (Jouaville *et al*. 1995, Wakai *et al*. 2013). In this regard, the protein MICU1 (mitochondrial calcium uptake 1) plays a principal role for Ca^{2+} uptake into the mitochondrial matrix (Perocchi *et al*. 2010). Interestingly, when mitochondrial function was uncoupled by either the protonophore FCCP (carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone) or AMA (antimycin A), both sperm and Sr^{2+}-induced Ca^{2+} transients were disrupted in mouse oocytes, resulting in a sustained and long Ca^{2+} increase, which resulted in apoptosis (Liu *et al*. 2001).

To this point, we have discussed the physiology associated with single transients and oscillatory Ca^{2+} signaling using *Xenopus* and mouse oocytes as reference models. The mechanisms mediating oocyte activation in both species show particular similarities with several findings demonstrating the role of sperm in triggering Ca^{2+} changes and the imperative role of the oocyte's machinery in modulating and shaping the final Ca^{2+} signature. The capability of oocytes in integrating distinct Ca^{2+} signals during fertilization is particularly observed using strategies to artificially induce oocyte activation. In this regard, disparate Ca^{2+} responses have demonstrated their efficiency in allowing egg-to-embryo transition, particularly in mammals. The following section aims to discuss the mechanisms of action of diverse activating agents and their impact in the physiology of mammalian oocyte activation subsequently affecting embryonic development.

Ca^{2+} signaling for assisted oocyte activation (AOA)

Protocols used for AOA can be classified based on whether the mechanism evoking the Ca^{2+} trigger that promotes fertilization is mechanical, electrical or chemical. Apart from the various mechanisms of action of these protocols, the response can also differ between different species (Vanden Meerschaut *et al*. 2014*b*). The mechanical approach involves the introduction of the sperm into the oocyte followed by vigorous ooplasm aspiration and re-injection during ICSI. In this way, the Ca^{2+} introduced from the extracellular medium is sufficient to restore the fertilization rates to normal, generally assumed as >70% oocytes showing two pronuclei (Tesarik & Sousa 1995, Tesarik *et al*. 2002). Electrostimulation involves the application of a pulsed high-voltage electrical field, which modifies the plasma membrane lipid bilayer leading to the formation of pores, and therefore, allowing Ca^{2+} entry from the extracellular medium (Versieren *et al*. 2010, Vanden Meerschaut *et al*. 2014*b*). However, chemical artificial activation is the most used strategy among practitioners. Chemical activation agents are classified based on the Ca^{2+} response they elicit in mammalian oocytes: single Ca^{2+} transients, dynamic Ca^{2+} oscillations and oocyte activation independent of the initial Ca^{2+} trigger. Table 1 summarizes AOA protocols commonly applied in mice and human. The mechanisms of action of the different activation agents as well as their limitations and benefits are further discussed.

Agents inducing single Ca^{2+} transients

Ca^{2+} ionophores

Calcium ionophores such as ionomycin and calcimycin are two carboxylic antibiotics, synthesized by the Streptomyces bacterial species *Streptomyces conglobatus* and *Streptomyces chartreusensis*, respectively. Ca^{2+} ionophores confer high permeability to cell membranes allowing Ca^{2+} ions to penetrate through. Oocytes exposed to Ca^{2+} ionophores experience an increase of free intracytoplasmic Ca^{2+}, resulting from Ca^{2+} influx as well as from Ca^{2+} release from the intracellular stores, particularly the ER, as described in starfish (Vasilev *et al*. 2012); no data specific to mouse and human oocytes are currently available.

Table 1 Chemical-based AOA protocols used in mouse and human studies.

Activation agent	Species	Ca^{2+} response	Activation rate	Blastocyst formation rate	Protocol #1	Protocol #2	References
Ionomycin	Mouse	Single transient	73.0%	24.0%	10 µM for 10 min (2 exposures, 30 min interval)	2 µg/mL cytoD for 3 h	Heytens *et al.* (2008)[a,1]
			66.3%	71.0%	10 µM for 10 min (2 exposures, 30 min interval)	2 µg/mL cytoD for 4 h	Nikiforaki *et al.* (2016)[a,1]
			85.0%	71.0%	10 µM for 10 min (2 exposures, 30 min interval)	Mouse wobbler sperm	Nikiforaki *et al.* (2016)[a,1]
	Human		86.1%*	16.7%	10 µM for 6 min (1 exposure)	2 mM 6-DMAP for 3 h	de Fried (2008)[1]
			30.0%	nd	10 µM for 10 min (2 exposures, 30 min interval) + 0.1 mol/L $CaCl_2$	Human *globozoospermic* sperm	Nikiforaki *et al.* (2016)[2]
			74.2%	nd	10 µM for 10 min (2 exposures, 30 min interval)	Human sperm with low or complete failed fertilization	Vanden Meerschaut *et al.* (2012)[1]
Calcimycin	Mouse		76.2%	32.3%	5 µM for 5 min (1 exposure)	1 µg/mL cytoD for 5 h	Uranga *et al.* (1996)[b,1]
			15.1%	43.7%	*Ready-to-use* solution for 15 min (1 exposure)	2 µg/mL cytoD for 4 h	Nikiforaki *et al.* (2016)[a,1]
			0.0%	0.0%	*Ready-to-use* solution for 15 min (1 exposure)	Mouse wobbler sperm	Nikiforaki *et al.* (2016)[a,1]
	Human		56.7%	9.1%	5 µM for 5 min (1 exposure)	2 mM 6-DMAP for 3 h	Liu *et al.* (2014)[3]
			11.8%	nd	*Ready-to-use* for 15 min (1 exposure)	Human *globozoospermic* sperm	Nikiforaki *et al.* (2016)[2]
Ethanol	Mouse		95.7%	86.3%	8.6% for 5 min (1 exposure)	0.5 µg/mL cytoD for 5–7 h	Cuthbertson (1983)[c,1]
			89.0%	71.0%	7% for 7 min (1 exposure)	2 µg/mL cytoD for 2 h	Rogers *et al.* (2006)[c,1]
	Human		16%	nd	8% for 10 min (1 exposure)	nd	Winston *et al.* (1991)[3]
			0%	nd	7% for 8–12 min (1 exposure)	nd	Balakier and Casper (1993)[3]
			82.7%	14.3%	7% for 6 min (1 exposure)	Human donor sperm	Liu (2014)[2]
$SrCl_2$	Mouse	Oscillatory signaling	90.0%	71.0%	10 mM for 2 h (1 exposure)	2 µg/mL cytoD for 2 h	Rogers *et al.* (2006)[c,1]
			96.5%	91.9%	10 mM for 4 h (1 exposure)	2 µg/mL cytoD	Nikiforaki *et al.* (2016)[a,1]
	Human		61.7%	25.7%	10 mM for 1 h (1 exposure)	Human sperm with low or complete failed fertilization	Kim *et al.* (2014)[1]
			54.5%	4.8%	10 mM for 20 min (1 exposure)	2.5 mM 6-DMAP for 1.5 h	Liu *et al.* (2014)[3]
PLCζ	Mouse		78.0%	62.0%	cRNA injection: 0.02 mg/mL	2 µM cytoD for 4 h	Saunders *et al.* (2002)[c,1]
			nd	±60%	Human recombinant protein injection: (0.0167 mg/mL)	5 µg/mL cytoB for 6 h	Nomikos *et al.* (2013)[c,1]
	Human		75%+	22.2%	cRNA injection: 0.1 µg/mL	2 µM cytoD for 2 h	Rogers *et al.* (2004)[3]
Puromycin	Human	In absence of the initial Ca^{2+} trigger	46.0%	nd	10 µg/mL for 5–24 h (1 exposure)	/	De Sutter *et al.* (1992)[3]
			91.0%	nd	100 µg/mL for 7–8 h (1 exposure)	/	Balakier and Casper (1993)[3]
Roscovitine	Mouse		81.0%	nd	50 µM for 8 h (1 exposure)	Mouse sperm	Phillips *et al.* (2002)[d,1]
Cycloheximide	Mouse		90.0%	24.0%	20 µg/mL for 4 h (1 exposure)	2 µg/mL cytoD for 2 h	Rogers *et al.* (2006)[c,1]
TPEN	Mouse		>40%	27.3%	100 µM for 45 min (1 exposure)	*5 µg/mL cytoB for 6 h*	Suzuki *et al.* (2010)[a,1]
	Human		nd	nd	*50µM for 30min (1 exposure)*	/	Duncan *et al.* (2016)[2]

Activation agents are classified based on the induced Ca^{2+} response. Activation rate is evaluated at day 1 after oocyte activation. For mouse studies: % 2-cell embryos. For human studies: % 2 pronuclei (pn) (*) Activation rate as % 1 pronuclei. +Activation rate as % 2-cell embryos. Protocols describe (#1) concentration of the activation agent and time of exposure and repetitions, (#2) strategy used to maintain diploidy. Mouse strains: [a]B6D2F1, [b]OF1, [c]MF1, [d]C57xA2G [e]CF1. Source of oocytes: [1]in vivo matured MII oocytes [2]IVF/ICSI failed to fertilize [3]in vitro matured MII oocytes. (nd) non available data.

Ethanol

Similarly, ethanol evokes a single Ca^{2+} transient, presumably caused by altered membrane fluidity followed by Ca^{2+} leakage through the membrane (Rybouchkin *et al*. 1996). As with Ca^{2+} ionophores, the ethanol-induced Ca^{2+} flux occurs during the time that ethanol is applied and recovers after ethanol washout.

The Ca^{2+} changes induced by Ca^{2+} ionophores or ethanol have not been reported to result in spontaneous dynamic Ca^{2+} oscillations. Interestingly, both stimuli have been demonstrated to activate mice and human oocytes leading to high rates of oocyte activation and blastocyst formation (Table 1). However, their competence in inducing oocyte activation and supporting blastocyst formation varies, particularly in humans, as described in several studies (Vanden Meerschaut *et al*. 2014*b*).

Agents inducing oscillatory Ca^{2+} signaling

Thimerosal

The sulfhydryl reagent thimerosal has been proposed as an activating agent given its capacity to induce Ca^{2+}-induced Ca^{2+} oscillations, as shown in mouse oocytes (Mehlmann & Kline 1994). Thimerosal induces fertilization-like oscillations by oxidizing protein thiol groups at the IP3R. As a consequence, a sensitization of the receptor to cytosolic concentration of IP3 is observed (Swann 1991). Despite evoking Ca^{2+} oscillations, thimerosal is unable to mediate meiotic resumption alone, given the redox effect that exerts on the cytoskeleton protein, therefore, impeding the cell cycle progression (Alexandre *et al*. 2003). Interestingly, thimerosal in combination with the redox inhibitor dithiothreitol (DTT) allows oocyte activation in several mammalian species (Swann 1991, Herbert *et al*. 1995, Macháty *et al*. 1997, Deng & Shen 2000). Moreover, the capacity of thimerosal in supporting embryonic development has only been described in pigs (Macháty *et al*. 1997).

Strontium

Strontium (Sr^{2+}) is a divalent ion that is similar in size to Ca^{2+}. It has been shown that Sr^{2+} is able to replace Ca^{2+} for triggering somatic cellular responses such as the neurotransmitters release (Matthew 2009). Similarly, mouse oocyte activation is efficiently mediated in culture media devoid of $CaCl_2$ and supplemented with $SrCl_2$; in this case, oscillatory fluorescence events reflect both, Ca^{2+} and Sr^{2+} variations. $SrCl_2$ is the most efficient method for mouse oocyte activation, which leads to high rates of embryo cleavage and blastocyst formation (Rogers *et al*. 2006, Nikiforaki *et al*. 2016) (Table 1). In contrast, the efficiency of Sr^{2+} in activating human oocytes is still debatable (Yanagida *et al*. 2006, Kim *et al*. 2015). Several authors described the efficiency of Sr^{2+} in 'rescuing' human oocytes that failed to fertilize after ICSI (Kyono *et al*. 2008, Kim *et al*. 2014). On the contrary, other authors did not find that Sr^{2+} stimulated Ca^{2+} oscillations even after several hours of measurements (Rogers *et al*. 2004, Lu 2015). Recently, the transient receptor potential cation channel, subfamily V, member 3 (TRPV3) was proposed as a mediator of Ca^{2+} influx in mouse oocytes (Carvacho *et al*. 2013). Its function was tested using TRPV3 agonists, such as 2-aminoethoxydiphenylborane (2-APB) and carvacrol, which mediated entry of Ca^{2+} into the oocyte and induced oocyte activation in mouse studies. Additionally, oocytes from TRPV3 knockout mice failed to respond to the agonists, nor did they conduct Sr^{2+} influx during Sr^{2+}-mediated oocyte activation in mouse (Carvacho *et al*. 2013), leading to an activation failure. Interestingly, TRPV3 agonists (2-APB and carvacrol) can mediate Ca^{2+} influx into human oocytes (Lu 2015), suggesting that TRPV3 channels are functional in human oocytes. However, no oscillations were observed after 6-h exposure to $SrCl_2$. These findings demonstrate the need to understand the role of TRPV3 channels during fertilization and their real implication in mediating Sr^{2+}-induced activation in human oocytes.

Recombinant phospholipase C ζ (PLCζ)

The capacity of both, mouse and human PLCζ proteins to induce Ca^{2+} oscillations and subsequently oocyte activation have been demonstrated (Kouchi *et al*. 2004, Rogers *et al*. 2004). It is worth considering that, PLCζ being the physiological stimuli to induce oocyte activation, the Ca^{2+} response would be expected to result in viable embryos. In this regard, the use of PLCζ as an oocyte activation agent seems promising, particularly in cases where sperm is devoid of PLCζ, such as in globozoospemia (Escoffier *et al*. 2015) or in patients carrying punctual mutations in PLCζ gene (Nomikos *et al*. 2013, Sanusi *et al*. 2015).

Oocyte activation in absence of the initial Ca^{2+}-trigger

Oocyte activation occurs as an immediate consequence of the complete degradation of the MPF, cdk1-cyclin b protein complex responsible for keeping the oocyte in meiotic arrest (Shoji *et al*. 2006). Therefore, designing an AOA approach requires targeting MPF degradation. As described earlier, numerous strategies are based on promoting intracellular Ca^{2+} changes to prime the initiation of the oocyte activation. However, several approaches have demonstrated their capacity to disrupt MPF function in the absence of the initial Ca^{2+} trigger. CAMKII cRNA allowed oocyte activation in mouse (Knott *et al*. 2006), supporting further embryonic development. Further downstream of CaMKII activation, a prominent zinc ion (Zn^{2+}) efflux, known as the Zn^{2+} spark, occurs during the course of oocyte activation, particularly after Emi2 inactivation (Bernhardt *et al*. 2011). This

Zn^{2+} spark is required for cyclin B degradation and the subsequent meiotic release (Suzuki *et al*. 2010). Artificial Zn^{2+} sequestration, bypassing the Ca^{2+} mobilization and mediated by the specific Zn^{2+} chelator N,N,N′,N-tetrakis (2-pyridylmethyl)ethane-1,2-diamine (TPEN), allows oocyte activation in several mammalian species, including human (Suzuki *et al*. 2010, Lee *et al*. 2015, Duncan *et al*. 2016). In addition, full-term embryonic development was achieved in mice and pigs by using TPEN as the only agent for oocyte activation. Moreover, different protein synthesis inhibitors such as puromycin (De Sutter *et al*. 1992, Lu *et al*. 2006) and cycloheximide (Moses & Kline 1995, Rogers *et al*. 2006) have been shown to alleviate meiotic arrest by interrupting the continuous synthesis of cyclin B. Roscovitine, in turn, was shown to induce the meiotic release by direct inhibition of the cdk1 function (Phillips *et al*. 2002).

AOA: clinical applications

AOA is a prerequisite to perform certain methodologies such as somatic cell nuclear transfer (SCNT) (Campbell *et al*. 2007) or parthenogenesis. However, AOA is mainly used as a complementary ART procedure to overcome fertilization failure after ICSI (Heindryckx *et al*. 2008, Vanden Meerschaut *et al*. 2012), and the application of Ca^{2+} ionophores is the most widely used method. Moreover, cases experiencing recurrent molar pregnancies have been identified as a population that could benefit from AOA application, as suggested in a recent study describing aberrant Ca^{2+} patterns upon *in vitro* fertilization by sperm involved in partial hydatidiform moles (Nikiforaki *et al*. 2014*a*). In these cases, Ca^{2+} ionophores might restore the lack of a normal sperm-induced Ca^{2+} response and provide the oocyte with sufficient Ca^{2+} for the accomplishment of cellular events associated with the process of oocyte activation, in particular granule exocytosis to block polyspermy (Ducibella *et al*. 2002). AOA has been recently proposed as a strategy to improve embryonic development in cases experiencing pronuclear and embryonic arrest (Darwish & Magdi 2015, Ebner *et al*. 2015). This application is supported by several findings demonstrating that oocyte activation and subsequent embryonic development might be mediated by the total summation of Ca^{2+} spikes during the oscillation period (Ozil *et al*. 2005, Tóth *et al*. 2006). The benefit of AOA in restoring fertilization rates is more evident in cases with sperm activation deficiencies. However, it is worth noting that few of the cases studied thus far did not benefit from the treatment (Vanden Meerschaut *et al*. 2012), possibly due to an underling oocyte quality issue. In this respect, methods intended to overcome the oocyte-related fertilization failure after ICSI remain to be further explored (Yeste *et al*. 2016). Moreover, AOA strategies remarkably differ between IVF centers. As previously suggested by our group, the variability observed among protocols confounds the comparison of the efficiency of these methods (reviewed by Vanden Meerschaut *et al*. 2013). Therefore, randomized comparative studies would be of benefit to select the most appropriate AOA strategy, contributing in turn to a robust standardization of the method. In this respect, a recent study compared Ca^{2+} release after exposing mouse and human oocytes to the Ca^{2+} ionophores ionomycin and calcimycin (Nikiforaki *et al*. 2016). Ionomycin induced higher amplitudes of the Ca^{2+} transient than calcimycin. As a result, ionomycin also led to significantly higher activation rates. Moreover, ionomycin resulted in higher blastocyst formation rates as evaluated in mouse. It is worth noting that calcimycin failed to activate oocytes pre-injected with mouse wobbler sperm, an animal model used for the study of globozoospermia (Heytens *et al*. 2010).

Mice are a popular translation model for human oocyte activation and AOA protocols and are commonly used to study the mechanisms involved in oocyte activation in mammals. However, other species such as the horse could closer reflect the fertilization events observed in humans, given the similarity of equine and human Ca^{2+} signatures observed during oocyte activation (Leemans *et al*. 2015).

Limitations and benefits of AOA strategies

The oocyte, unlike other cell types in which the initiation of diverse cellular pathways occurs in response to a certain frequency of Ca^{2+} oscillations, is capable of integrating different types of Ca^{2+} signatures to successfully resume the cell cycle (Dupont *et al*. 2010). Methodologies that evade dynamic Ca^{2+} oscillations (e.g. Ca^{2+} ionophores) have been shown to activate mammalian oocytes and consequently, to stimulate the embryonic development, merely by inducing single Ca^{2+} transients (Ozil 1998). However, Ca^{2+} oscillations are essential to completely inactivate MPF as well as to impede its rebound (Ducibella *et al*. 2002, Tóth *et al*. 2006). Early and late embryo developmental events occurring during the process of oocyte activation, such as cortical granules exocytosis or the maternal mRNAs recruitment, are stimulated by a different number of Ca^{2+} transients (Ducibella *et al*. 2002). As a result, these cellular events require a lower number of Ca^{2+} oscillations for their initiation (e.g. 4 or 8 transients) than for their completion (e.g. 24 transients). Moreover, the impact of Ca^{2+} signature patterns on the subsequent embryonic development, ranging from the first embryonic interphase at the pronuclear stage to the blastulation, has been repeatedly described (Ozil *et al*. 2005, 2006, Tóth *et al*. 2006). Manipulating the Ca^{2+} oscillatory pattern during oocyte activation did not impair the activation or the blastocyst formation rates in mouse. However, when an early cessation of Ca^{2+} oscillations was provoked, gene expression profiles showed that preimplantation potential was

compromised (Ozil *et al*. 2006). Furthermore, blastocysts resulting from a Ca^{2+} hyperstimulation revealed gene expression profiles associated with post-implantation failure (Ozil *et al*. 2006). The impact of the absence of an initial Ca^{2+} trigger to induce oocyte activation has also been evaluated. Oocytes stimulated by agents such as cycloheximide (Rogers *et al*. 2006) or TPEN (Suzuki *et al*. 2010) showed normal activation rates, in the absence of Ca^{2+} rises. However, their efficiency in supporting subsequent embryonic development to blastocyst stage was remarkably low (Fig. 2). Interestingly, the additional application of a treatment that promotes cytoplasmic Ca^{2+} increase such as ethanol that causes single Ca^{2+} transients, or $SrCl_2$, that causes Ca^{2+} oscillations, restored embryonic development rates to normal (Rogers *et al*. 2006). These findings highlight the prominent role of Ca^{2+} in fertilization and particularly in supporting embryonic development (Fig. 2). Following the hypothesis in which the total amount of Ca^{2+} released would determine for a minimum threshold required to achieve oocyte activation (Ozil *et al*. 2005, Tóth *et al*. 2006), it is not difficult to understand that the single Ca^{2+} transients induced by Ca^{2+} ionophores are sufficient to trigger oocyte activation. In support, a predictive minimal mathematical model reflected the capacity of single Ca^{2+} transients to induce meiotic alleviation in mammals (Dupont *et al*. 2010).

As described earlier, Ca^{2+} ionophores use is the most common AOA strategy used in ART. Besides the substantial cellular stress that Ca^{2+} ionophores might exert by altering membrane permeability, safety concerns remain regarding the induction of a single Ca^{2+} transient which differs remarkably from the physiological oscillatory Ca^{2+} response (Santella & Dale 2015, van Blerkom *et al*. 2015). Accumulating evidence supports the biosafety of ionomycin as an activating agent. First, high oocyte survival rates are observed following AOA in mouse (Heytens *et al*. 2008) and in human (Heindryckx *et al*. 2008) oocytes. Moreover, ionomycin did not increase the incidence of meiotic errors of maternal origin in human oocytes (Capalbo *et al*. 2016). Most importantly, the follow-up studies of children born after AOA support the safety of this methodology (D'haeseleer *et al*. 2014, Vanden Meerschaut *et al*. 2014*a*, Miller *et al*. 2016). Together, these data endorse the readiness of AOA for clinical applications (Ebner & Montag 2016). However, defining a proper indication requires further investigation (van Blerkom *et al*. 2015). Diagnostic tools to identify cases that could benefit from AOA are needed to help clinicians give appropriate medical advice. To this end, heterologous ICSI was introduced as a valuable approach to determine whether an oocyte-related or sperm-related factor is causing the fertilization failure (Rybouchkin *et al*. 1995, Heindryckx *et al*. 2005). Accordingly, the mouse oocyte activation test (MOAT) is an efficient assay to reveal the presence of sperm-related activation deficiencies. The MOAT is used for patients experiencing failed fertilization after ICSI and involves injection of human sperm into mouse oocytes, to classify the sperm activation capacity

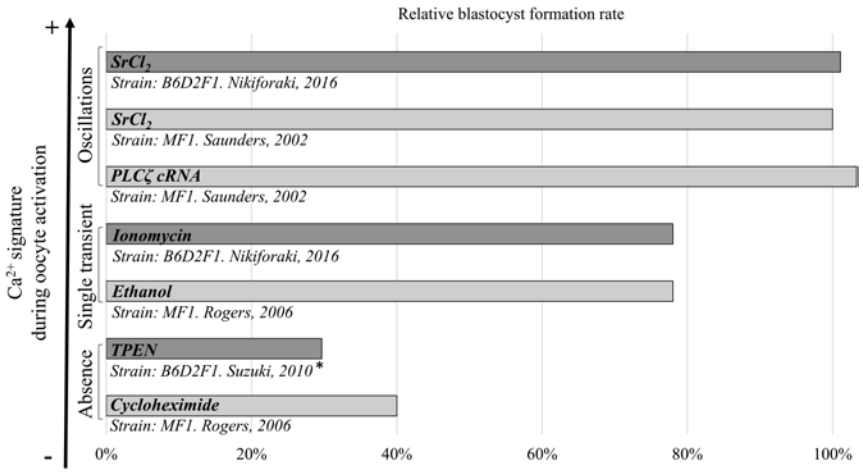

Figure 2 Schematic diagram of impact of Ca^{2+} signature during AOA on blastocyst formation rate. Data represent the efficiency of different AOA strategies to support blastocyst formation compared to *in vivo*-fertilized oocytes (further cultured *in vitro* after zygote collection). AOA strategies that supply the oocyte with Ca^{2+} during activation reflect similar capacity to support blastocyst formation than oocytes that were fertilized *in vivo*. This capacity is affected by the absence of Ca^{2+} during activation, as observed in studies that used TPEN and cyclohexamide as activation factors. The figure has been designed based on available data and includes only mouse studies. Normalization has been performed based on the blastocyst formation rate (%) observed after *in vivo* fertilization. Reference data were obtained from studies that used the same mouse strain and, where possible, were performed in the same lab. Experiments with MF1 mice (light gray), are normalized to blastocyst formation rate obtained by Saunders *et al*. (2002), which was 87%. Experiments with B6D2F1 mice (dark gray) are normalized to blastocyst formation rate obtained by Neupane *et al*. (2014), which was 91%. Only blastocyst formation rate reported after the use of TPEN was obtained in same mouse strain (B6D2F1) but in a different lab.

into one of three categories (Heindryckx *et al*. 2005). The activation capacity is defined by observing the percentage of 2-cell embryos 24 h after ICSI and results are compared to a fertile control. MOAT groups range from low-to-high activation potential. MOAT group 1 (0–20% of 2-cell embryos 24 h after ICSI) includes patients with a sperm-related activation deficiency, such as cases with globozoospermia (Heytens *et al*. 2010) or carriers of mutations in the PLCζ gene (Heytens *et al*. 2009, Kashir *et al*. 2012). MOAT 2 (21–84%) includes patients showing diminished activation capacity and MOAT 3 (>85%) includes patients indicating a normal activation capacity, comparable to a positive control and consequently pointing more to an oocyte-related activation deficiency (Heindryckx *et al*. 2005). Additionally, further investigation revealed an interesting correlation between the Ca^{2+} oscillatory patterns and the MOAT groups. The outcome obtained by the mouse oocyte calcium analysis (MOCA) defined a finer classification on the human sperm activation potential and highlighted the importance of studying Ca^{2+} patterns in cases experiencing ICSI failures. Both heterologous tests, MOAT and MOCA, are valuable approaches to use for in medical counseling (Vanden Meerschaut *et al*. 2013). Considering that activation potential is directly related to PLCζ properties (Nomikos *et al*. 2014*a*), the MOAT and MOCA tests can be considered as useful tools for evaluating PLCζ functionality. However, it is worth noting that there are interspecies differences, since human PLCζ shows greater Ca^{2+} oscillation-inducing potency than the mouse PLCζ in conspecific oocytes (Cox *et al*. 2002, Nomikos *et al*. 2014*b*). Performing Ca^{2+} analysis using human oocytes to measure human sperm activation capacity has been proposed as an add-on methodology to the heterologous tests (Ferrer-Buitrago 2016). However, the scarcity of human oocytes donated for research purposes is a major limitation.

Conclusion

The process of oocyte activation is firmly associated with a specialized Ca^{2+} signal, which occurs as single Ca^{2+} transients or Ca^{2+} oscillations depending on the species (Stricker 1999). The prominent role of the oocyte's Ca^{2+} machinery in modulating the Ca^{2+} signature is clear from *Xenopus* sperm extract studies, which provoke a single Ca^{2+} transient in *Xenopus* oocytes but induced Ca^{2+} oscillations in mouse oocytes (Dong *et al*. 2000). In this regard, the regulation of the oocyte's machinery plays a crucial role in modulating Ca^{2+} profile in preparation to the egg-to-embryo transition. Moreover, mammalian oocytes can respond to artificially induced single Ca^{2+} transients or strategies, which mediate meiotic alleviation in the absence of the initial Ca^{2+} trigger, both resulting in oocyte activation (Table 1). This is probably because molecular events, in particular, the activation of effectors such as CAMKII, occur in response to a minimum threshold of Ca^{2+} levels (Ozil *et al*. 2005, Tóth *et al*. 2006). Moreover, MPF degradation and subsequent meiotic alleviation can occur bypassing the increase of Ca^{2+} or presence of oscillations, in response to the destabilization of proteins responsible of the meiotic arrest. However, the use of strategies that induce Ca^{2+} oscillatory responses probably would show further benefits as they would more closely mimic the physiological situation. The importance of Ca^{2+} oscillations in achieving high blastocyst formation rates have been demonstrated in certain mammalian species (Ozil 1998). Moreover, specific Ca^{2+} oscillatory patterns have an impact on events required for full-term mammalian embryonic development, both early (e.g. oocyte activation) (Ducibella *et al*. 2002, Tóth *et al*. 2006, Ducibella & Fissore 2008) and late (e.g. post-implantation viability) (Ozil *et al*. 2006). Methods to intervene more accurately the frequency of Ca^{2+} oscillations would allow discerning whether the presence of Ca^{2+} oscillations has additional impact in cellular processes occurring during the egg-to-embryo transition, such as the late events of parental chromatin remodeling: chromatin decondensation within the male pronucleus and the import of nuclear proteins (McLay & Clarke 2003). Despite the benefits reported on the presence of Ca^{2+} oscillations for subsequent embryonic development, Ca^{2+} oscillatory responses are not regularly used for clinical applications in the human. In this regard, Ca^{2+} ionophores, inducing single Ca^{2+} transients, are nowadays mostly used for AOA in ART, in particular to overcome fertilization failure after ICSI. To our knowledge and experience, the use of Ca^{2+} ionophores is the most efficient AOA strategy currently known for application in the human. Accumulating evidence supports their efficiency in achieving embryonic development and pregnancies to term. However, developing strategies to artificially induce Ca^{2+} oscillatory activity in human oocytes are urgently needed. Given the inconsistency of the data reported after the use of Sr^{2+}in human, the intracytoplasmic injection of human PLCζ represents a promising AOA approach to achieve a more physiological Ca^{2+} response, which may have substantial clinical impact (Swann & Lai 2016). Mouse studies have reported on the efficiency of PLCζ cRNA to induce oocyte activation (Cox *et al*. 2002, Yu *et al*. 2008). The minimum concentration of human PLCζ associated with mouse oocyte activation was calculated as 1 fg after the injection of a solution containing human PLCζ cRNA concentrations of 0.05–0.05 µg/µL (Yu *et al*. 2008). Moreover, the direct injection of 80 fg purified recombinant human PLCζ protein showed its potential to rescue oocyte activation failure in mouse oocytes pre-injected with cRNA encoding for mutant forms of PLCζ (Nomikos *et al*. 2013). Although oocyte activation occurs over a wide range of PLCζ levels, embryonic development is supported within a narrow window of protein concentrations (Yu *et al*. 2008), in correlation

with the total number of Ca^{2+} oscillations observed during oocyte activation. The study performed by Yu and coworkers (Yu *et al*. 2008) evaluated mouse blastocyst formation potential in association with PLCζ levels expressed as counts of luminescence per second (cps). With 1 cps corresponding to ~250 fg, higher blastocyst formation rates were achieved within the range of 0.12–2.5 cps, thus from 30 to 625 fg of human PLCζ. Of note, low and high levels of PLCζ were associated with low oocyte activation potential and embryo development arrest, respectively (Yu *et al*. 2008). Moreover, the direct injection of recombinant human PLCζ at a concentration of 80 fg allowed mouse blastocyst formation in a proposed prototype for male infertility based on failed fertilization observed after the injection of PLCζ cRNA encoding known point mutations (Kashir *et al*. 2012, Nomikos *et al*. 2013). A definite protocol describing whether exogenous PLCζ would induce a real benefit for human oocyte activation and embryo development has not been yet established. However, the activation capacity of human PLCζ cRNA (Rogers *et al*. 2004, Nomikos *et al*. 2013) has also been demonstrated in human oocytes. As observed in mouse studies, although oocyte activation is associated with a broad range of PLCζ cRNA concentrations (10–0.1 µg/mL), higher blastocyst formation rates were obtained after the injection of PLCζ cRNA at the lowest concentration (0.1 µg/mL) (Rogers *et al*. 2004). Further research is required to standardize this methodology and evaluate long-term safety in offspring.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

This work was supported by the Flemish fund for scientific research (FWO-Vlaanderen, grant number G060615N), the Interuniversity Attraction Poles Program (Belgian Science Policy) and Ghent University.

Authors' contribution statement

M F B and D B were responsible for the literature search and making the table. M F B was responsible for making the figures. M F B, D B, L L and B H were responsible for writing of the manuscript. P D S, L L and B H were responsible for the supervision and the approval of this manuscript.

Acknowledgements

The authors would like to thank Dr Fiona Dunlevy (Axcience) for providing the editorial support.

References

Ajduk A, Małagocki A & Maleszewski M 2008 Cytoplasmic maturation of mammalian oocytes: development of a mechanism responsible for sperm-induced Ca2+ oscillations. *Reproductive Biology* **8** 3–22. (https://doi.org/10.1016/S1642-431X(12)60001-1)

Alexandre H, Delsinne V & Goval JJ 2003 The thiol reagent, thimerosal, irreversibly inhibits meiosis reinitiation in mouse oocyte when applied during a very early and narrow temporal window: a pharmacological analysis. *Molecular Reproduction and Development* **65** 454–461. (https://doi.org/10.1002/mrd.10319)

Ayabe T, Kopf GS & Schultz RM 1995 Regulation of mouse egg activation: presence of ryanodine receptors and effects of microinjected ryanodine and cyclic ADP ribose on uninseminated and inseminated eggs. *Development* **121** 2233–2244.

Backs J, Stein P, Backs T, Duncan FE, Grueter CE, McAnally J, Qi X, Schultz RM & Olson EN 2010 The gamma isoform of CaM kinase II controls mouse egg activation by regulating cell cycle resumption. *PNAS* **107** 81–86. (https://doi.org/10.1073/pnas.0912658106)

Balakier H & Casper RF 1993 Experimentally induced parthenogenetic activation of human oocytes. *Human Reproduction* **8** 740–743. (https://doi.org/10.1093/oxfordjournals.humrep.a138132)

Bernhardt ML, Kim AM, O'Halloran TV & Woodruff TK 2011 Zinc requirement during meiosis I-meiosis II transition in mouse oocytes is independent of the MOS-MAPK pathway. *Biology of Reproduction* **84** 526–536. (https://doi.org/10.1095/biolreprod.110.086488)

Berridge MJ, Lipp P & Bootman MD 2000 The versatility and universality of calcium signalling. *Nature Reviews Molecular Cell Biology* **1** 11–21. (https://doi.org/10.1038/35036035)

Bezprozvanny I 2005 The inositol 1,4,5-trisphosphate receptors. *Cell Calcium* **38** 261–272. (https://doi.org/10.1016/j.ceca.2005.06.030)

Bootman MD, Collins TJ, Peppiatt CM, Prothero LS, MacKenzie L, De Smet P, Travers M, Tovey SC, Seo JT, Berridge MJ *et al*. 2001 Calcium signalling – an overview. *Seminars in Cell and Developmental Biology* **12** 3–10. (https://doi.org/10.1006/scdb.2000.0211)

Bootman MD, Berridge MJ & Roderick HL 2002 Calcium signalling: more messengers, more channels, more complexity. *Current Biology* **12** R563–R565. (https://doi.org/10.1016/S0960-9822(02)01055-2)

Brandman O, Liou J, Park WS & Meyer T 2007 STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca2+ levels. *Cell* **131** 1327–1339. (https://doi.org/10.1016/j.cell.2007.11.039)

Cahalan MD 2009 STIMulating store-operated Ca(2+) entry. *Nature Cell Biology* **11** 669–677. (https://doi.org/10.1038/ncb0609-669)

Campbell KH, Fisher P, Chen WC, Choi I, Kelly RD, Lee JH & Xhu J 2007 Somatic cell nuclear transfer: past, present and future perspectives. *Theriogenology* **68** (Supplement 1) S214–S231. (https://doi.org/10.1016/j.theriogenology.2007.05.059)

Capalbo A, Ottolini CS, Griffin DK, Ubaldi FM, Handyside AH & Rienzi L 2016 Artificial oocyte activation with calcium ionophore does not cause a widespread increase in chromosome segregation errors in the second meiotic division of the oocyte. *Fertility and Sterility* **105** 807.e802–814.e802. (https://doi.org/10.1016/j.fertnstert.2015.11.017)

Carrasco S & Meyer T 2011 STIM proteins and the endoplasmic reticulum-plasma membrane junctions. *Annual Review of Biochemistry* **80** 973–1000. (https://doi.org/10.1146/annurev-biochem-061609-165311)

Carroll J 2000 Na+-Ca2+ exchange in mouse oocytes: modifications in the regulation of intracellular free Ca2+ during oocyte maturation. *Journal of Reproduction and Fertility* **118** 337–342. (https://doi.org/10.1530/jrf.0.1180337)

Carvacho I, Lee HC, Fissore RA & Clapham DE 2013 TRPV3 channels mediate strontium-induced mouse-egg activation. *Cell Reports* **5** 1375–1386. (https://doi.org/10.1016/j.celrep.2013.11.007)

Cheon B, Lee HC, Wakai T & Fissore RA 2013 Ca2+ influx and the store-operated Ca2+ entry pathway undergo regulation during mouse oocyte maturation. *Molecular Biology of the Cell* **24** 1396–1410. (https://doi.org/10.1091/mbc.E13-01-0065)

Courjaret R, Dib M & Machaca K 2017 Store-operated Ca(2+) entry in oocytes modulate the dynamics of ip3-dependent Ca(2+) release from oscillatory to tonic. *Journal of Cellular Physiology* **232** 1095–1103. (https://doi.org/10.1002/jcp.25513)

Coward K, Ponting CP, Zhang N, Young C, Huang CJ, Chou CM, Kashir J, Fissore RA & Parrington J 2011 Identification and functional analysis of

an ovarian form of the egg activation factor phospholipase C zeta (PLCζ) in pufferfish. *Molecular Reproduction and Development* **78** 48–56. (https://doi.org/10.1002/mrd.21262)

Cox LJ, Larman MG, Saunders CM, Hashimoto K, Swann K & Lai FA 2002 Sperm phospholipase Czeta from humans and cynomolgus monkeys triggers Ca2+ oscillations, activation and development of mouse oocytes. *Reproduction* **124** 611–623. (https://doi.org/10.1530/rep.0.1240611)

Cuthbertson KS 1983 Parthenogenetic activation of mouse oocytes in vitro with ethanol and benzyl alcohol. *Journal of Experimental Zoology* **226** 311–314. (https://doi.org/10.1002/jez.1402260217)

D'haeseleer E, Vanden Meerschaut F, Bettens K, Luyten A, Gysels H, Thienpont Y, De Witte G, Heindryckx B, Oostra A, Roeyers H *et al*. 2014 Language development of children born following intracytoplasmic sperm injection (ICSI) combined with assisted oocyte activation (AOA). *International Journal of Language and Communication Disorders* **49** 702–709. (https://doi.org/10.1111/1460-6984.12100)

Darwish E & Magdi Y 2015 A preliminary report of successful cleavage after calcium ionophore activation at ICSI in cases with previous arrest at the pronuclear stage. *Reproductive BioMedicine Online* **31** 799–804. (https://doi.org/10.1016/j.rbmo.2015.08.012)

de Fried EP, Ross P, Zang G, Divita A, Cunniff K, Denaday F, Salamone D, Kiessling A & Cibelli J 2008 Human parthenogenetic blastocysts derived from noninseminated cryopreserved human oocytes. *Fertility and Sterility* **89** 943–947. (https://doi.org/10.1016/j.fertnstert.2007.04.045)

De Sutter P, Dozortsev D, Cieslak J, Wolf G, Verlinsky Y & Dyban A 1992 Parthenogenetic activation of human oocytes by puromycin. *Journal of Assisted Reproduction and Genetics* **9** 328–337. (https://doi.org/10.1007/BF01203955)

Deguchi R, Shirakawa H, Oda S, Mohri T & Miyazaki S 2000 Spatiotemporal analysis of Ca(2+) waves in relation to the sperm entry site and animal-vegetal axis during Ca(2+) oscillations in fertilized mouse eggs. *Developmental Biology* **218** 299–313. (https://doi.org/10.1006/dbio.1999.9573)

Deng MQ & Shen SS 2000 A specific inhibitor of p34(cdc2)/cyclin B suppresses fertilization-induced calcium oscillations in mouse eggs. *Biology of Reproduction* **62** 873–878. (https://doi.org/10.1095/biolreprod62.4.873)

Dong JB, Tang TS & Sun FZ 2000 Xenopus and chicken sperm contain a cytosolic soluble protein factor which can trigger calcium oscillations in mouse eggs. *Biochemical and Biophysical Research Communications* **268** 947–951. (https://doi.org/10.1006/bbrc.2000.2218)

Ducibella T & Fissore R 2008 The roles of Ca2+, downstream protein kinases, and oscillatory signaling in regulating fertilization and the activation of development. *Developmental Biology* **315** 257–279. (https://doi.org/10.1016/j.ydbio.2007.12.012)

Ducibella T, Huneau D, Angelichio E, Xu Z, Schultz RM, Kopf GS, Fissore R, Madoux S & Ozil JP 2002 Egg-to-embryo transition is driven by differential responses to Ca(2+) oscillation number. *Developmental Biology* **250** 280–291. (https://doi.org/10.1006/dbio.2002.0788)

Dumollard R, Marangos P, Fitzharris G, Swann K, Duchen M & Carroll J 2004 Sperm-triggered [Ca2+] oscillations and Ca2+ homeostasis in the mouse egg have an absolute requirement for mitochondrial ATP production. *Development* **131** 3057–3067. (https://doi.org/10.1242/dev.01181)

Duncan FE, Que EL, Zhang N, Feinberg EC, O'Halloran TV & Woodruff TK 2016 The zinc spark is an inorganic signature of human egg activation. *Scientific Reports* **6** 24737. (https://doi.org/10.1038/srep24737)

Dupont G, Heytens E & Leybaert L 2010 Oscillatory Ca2+ dynamics and cell cycle resumption at fertilization in mammals: a modelling approach. *International Journal of Developmental Biology* **54** 655–665. (https://doi.org/10.1387/ijdb.082845gd)

Ebner T & Montag M 2016 Artificial oocyte activation: evidence for clinical readiness. *Reproductive BioMedicine Online* **32** 271–273. (https://doi.org/10.1016/j.rbmo.2015.12.004)

Ebner T, Oppelt P, Wöber M, Staples P, Mayer RB, Sonnleitner U, Bulfon-Vogl S, Gruber I, Haid AE & Shebl O 2015 Treatment with Ca2+ ionophore improves embryo development and outcome in cases with previous developmental problems: a prospective multicenter study. *Human Reproduction* **30** 97–102. (https://doi.org/10.1093/humrep/deu285)

El-Jouni W, Haun S & Machaca K 2008 Internalization of plasma membrane Ca2+-ATPase during Xenopus oocyte maturation. *Developmental Biology* **324** 99–107. (https://doi.org/10.1016/j.ydbio.2008.09.007)

Escoffier J, Yassine S, Lee HC, Martinez G, Delaroche J, Coutton C, Karaouzene T, Zouari R, Metzler-Guillemain C, Pernet-Gallay K *et al*. 2015 Subcellular localization of phospholipase Czeta in human sperm and its absence in DPY19L2-deficient sperm are consistent with its role in oocyte activation. *Molecular Human Reproduction* **21** 157–168. (https://doi.org/10.1093/molehr/gau098)

Ferrer-Buitrago M 2016 Predictive value of human calcium pattern analysis on the benefit of assisted oocyte activation in cases of failed fertilization after ICSI. Abstract Number: i234. Presented at the 32nd ESHRE Annual Meeting, Helsinki, Finland.

Fissore RA, Longo FJ, Anderson E, Parys JB & Ducibella T 1999 Differential distribution of inositol trisphosphate receptor isoforms in mouse oocytes. *Biology of Reproduction* **60** 49–57. (https://doi.org/10.1095/biolreprod60.1.49)

Fissore RA, Kurokawa M, Knott J, Zhang M & Smyth J 2002 Mechanisms underlying oocyte activation and postovulatory ageing. *Reproduction* **124** 745–754. (https://doi.org/10.1530/rep.0.1240745)

Gonzalez-Garcia JR, Machaty Z, Lai FA & Swann K 2013 The dynamics of PKC-induced phosphorylation triggered by Ca2+ oscillations in mouse eggs. *Journal of Cellular Physiology* **228** 110–119. (https://doi.org/10.1002/jcp.24110)

Goud PT, Goud AP, Van Oostveldt P & Dhont M 1999 Presence and dynamic redistribution of type I inositol 1,4,5-trisphosphate receptors in human oocytes and embryos during in-vitro maturation, fertilization and early cleavage divisions. *Molecular Human Reproduction* **5** 441–451. (https://doi.org/10.1093/molehr/5.5.441)

Halet G 2004 PKC signaling at fertilization in mammalian eggs. *Biochimica et Biophysica Acta* **1742** 185–189. (https://doi.org/10.1016/j.bbamcr.2004.09.012)

Halet G, Tunwell R, Balla T, Swann K & Carroll J 2002 The dynamics of plasma membrane PtdIns(4,5)P(2) at fertilization of mouse eggs. *Journal of Cell Science* **115** 2139–2149.

Harada Y, Kawazoe M, Eto Y, Ueno S & Iwao Y 2011 The Ca2+ increase by the sperm factor in physiologically polyspermic newt fertilization: its signaling mechanism in egg cytoplasm and the species-specificity. *Developmental Biology* **351** 266–276. (https://doi.org/10.1016/j.ydbio.2011.01.003)

Heindryckx B, Van der Elst J, De Sutter P & Dhont M 2005 Treatment option for sperm- or oocyte-related fertilization failure: assisted oocyte activation following diagnostic heterologous ICSI. *Human Reproduction* **20** 2237–2241. (https://doi.org/10.1093/humrep/dei029)

Heindryckx B, De Gheselle S, Gerris J, Dhont M & De Sutter P 2008 Efficiency of assisted oocyte activation as a solution for failed intracytoplasmic sperm injection. *Reproductive BioMedicine Online* **17** 662–668. (https://doi.org/10.1016/S1472-6483(10)60313-6)

Herbert M, Murdoch AP & Gillespie JI 1995 The thiol reagent, thimerosal induces intracellular calcium oscillations in mature human oocytes. *Human Reproduction* **10** 2183–2186. (https://doi.org/10.1093/oxfordjournals.humrep.a136265)

Heytens E, Soleimani R, Lierman S, De Meester S, Gerris J, Dhont M, Van der Elst J & De Sutter P 2008 Effect of ionomycin on oocyte activation and embryo development in mouse. *Reproductive BioMedicine Online* **17** 764–771. (https://doi.org/10.1016/S1472-6483(10)60403-8)

Heytens E, Parrington J, Coward K, Young C, Lambrecht S, Yoon SY, Fissore RA, Hamer R, Deane CM, Ruas M *et al*. 2009 Reduced amounts and abnormal forms of phospholipase C zeta (PLCzeta) in spermatozoa from infertile men. *Human Reproduction* **24** 2417–2428. (https://doi.org/10.1093/humrep/dep207)

Heytens E, Schmitt-John T, Moser JM, Jensen NM, Soleimani R, Young C, Coward K, Parrington J & De Sutter P 2010 Reduced fertilization after ICSI and abnormal phospholipase C zeta presence in spermatozoa from the wobbler mouse. *Reproductive BioMedicine Online* **21** 742–749. (https://doi.org/10.1016/j.rbmo.2010.07.006)

Hogan PG, Lewis RS & Rao A 2010 Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annual Review of Immunology* **28** 491–533. (https://doi.org/10.1146/annurev.immunol.021908.132550)

Ito J, Yoon SY, Lee B, Vanderheyden V, Vermassen E, Wojcikiewicz R, Alfandari D, De Smedt H, Parys JB & Fissore RA 2008 Inositol 1,4,5-trisphosphate receptor 1, a widespread Ca2+ channel, is a novel

substrate of polo-like kinase 1 in eggs. *Developmental Biology* **320** 402–413. (https://doi.org/10.1016/j.ydbio.2008.05.548)

Ito J, Parrington J & Fissore RA 2011 PLCζ and its role as a trigger of development in vertebrates. *Molecular Reproduction and Development* **78** 846–853. (https://doi.org/10.1002/mrd.21359)

Jouaville LS, Ichas F, Holmuhamedov EL, Camacho P & Lechleiter JD 1995 Synchronization of calcium waves by mitochondrial substrates in Xenopus laevis oocytes. *Nature* **377** 438–441. (https://doi.org/10.1038/377438a0)

Kashir J, Konstantinidis M, Jones C, Heindryckx B, De Sutter P, Parrington J, Wells D & Coward K 2012 Characterization of two heterozygous mutations of the oocyte activation factor phospholipase C zeta (PLCζ) from an infertile man by use of minisequencing of individual sperm and expression in somatic cells. *Fertility and Sterility* **98** 423–431. (https://doi.org/10.1016/j.fertnstert.2012.05.002)

Kashir J, Deguchi R, Jones C, Coward K & Stricker SA 2013 Comparative biology of sperm factors and fertilization-induced calcium signals across the animal kingdom. *Molecular Reproduction and Development* **80** 787–815. (https://doi.org/10.1002/mrd.22222)

Kim JW, Kim SD, Yang SH, Yoon SH, Jung JH & Lim JH 2014 Successful pregnancy after SrCl2 oocyte activation in couples with repeated low fertilization rates following calcium ionophore treatment. *Systems Biology in Reproductive Medicine* **60** 177–182. (https://doi.org/10.3109/19396368.2014.900832)

Kim JW, Yang SH, Yoon SH, Kim SD, Jung JH & Lim JH 2015 Successful pregnancy and delivery after ICSI with artificial oocyte activation by calcium ionophore in in-vitro matured oocytes: a case report. *Reproductive BioMedicine Online* **30** 373–377. (https://doi.org/10.1016/j.rbmo.2014.11.014)

Kline D, Mehlmann L, Fox C & Terasaki M 1999 The cortical endoplasmic reticulum (ER) of the mouse egg: localization of ER clusters in relation to the generation of repetitive calcium waves. *Developmental Biology* **215** 431–442. (https://doi.org/10.1006/dbio.1999.9445)

Knott JG, Gardner AJ, Madgwick S, Jones KT, Williams CJ & Schultz RM 2006 Calmodulin-dependent protein kinase II triggers mouse egg activation and embryo development in the absence of Ca2+ oscillations. *Developmental Biology* **296** 388–395. (https://doi.org/10.1016/j.ydbio.2006.06.004)

Kouchi Z, Fukami K, Shikano T, Oda S, Nakamura Y, Takenawa T & Miyazaki S 2004 Recombinant phospholipase Czeta has high Ca2+ sensitivity and induces Ca2+ oscillations in mouse eggs. *Journal of Biological Chemistry* **279** 10408–10412. (https://doi.org/10.1074/jbc.M313801200)

Kume S, Muto A, Aruga J, Nakagawa T, Michikawa T, Furuichi T, Nakade S, Okano H & Mikoshiba K 1993 The Xenopus IP3 receptor: structure, function, and localization in oocytes and eggs. *Cell* **73** 555–570. (https://doi.org/10.1016/0092-8674(93)90142-D)

Kyono K, Kumagai S, Nishinaka C, Nakajo Y, Uto H, Toya M, Sugawara J & Araki Y 2008 Birth and follow-up of babies born following ICSI using SrCl2 oocyte activation. *Reproductive BioMedicine Online* **17** 53–58. (https://doi.org/10.1016/S1472-6483(10)60293-3)

Lee B, Vermassen E, Yoon SY, Vanderheyden V, Ito J, Alfandari D, De Smedt H, Parys JB & Fissore RA 2006 Phosphorylation of IP3R1 and the regulation of [Ca2+]i responses at fertilization: a role for the MAP kinase pathway. *Development* **133** 4355–4365. (https://doi.org/10.1242/dev.02624)

Lee K, Wang C & Machaty Z 2012 STIM1 is required for Ca2+ signaling during mammalian fertilization. *Developmental Biology* **367** 154–162. (https://doi.org/10.1016/j.ydbio.2012.04.028)

Lee K, Davis A, Zhang L, Ryu J, Spate LD, Park KW, Samuel MS, Walters EM, Murphy CN, Machaty Z *et al*. 2015 Pig oocyte activation using a Zn^{2+} chelator, TPEN. *Theriogenology* **84** 1024–1032. (https://doi.org/10.1016/j.theriogenology.2015.05.036)

Leemans B, Gadella BM, Stout TA, Heras S, Smits K, Ferrer-Buitrago M, Claes E, Heindryckx B, De Vos WH, Nelis H *et al*. 2015 Procaine induces cytokinesis in horse oocytes via a pH-dependent mechanism. *Biology of Reproduction* **93** 23.

Lewis RS 2007 The molecular choreography of a store-operated calcium channel. *Nature* **446** 284–287. (https://doi.org/10.1038/nature05637)

Liou J, Fivaz M, Inoue T & Meyer T 2007 Live-cell imaging reveals sequential oligomerization and local plasma membrane targeting of stromal interaction molecule 1 after Ca2+ store depletion. *PNAS* **104** 9301–9306. (https://doi.org/10.1073/pnas.0702866104)

Liu L, Hammar K, Smith PJ, Inoue S & Keefe DL 2001 Mitochondrial modulation of calcium signaling at the initiation of development. *Cell Calcium* **30** 423–433. (https://doi.org/10.1054/ceca.2001.0251)

Liu Y, Han XJ, Liu MH, Wang SY, Jia CW, Yu L, Ren G, Wang L & Li W 2014 Three-day-old human unfertilized oocytes after in vitro fertilization/intracytoplasmic sperm injection can be activated by calcium ionophore a23187 or strontium chloride and develop to blastocysts. *Cell Reprogram* **16** 276–280. (https://doi.org/10.1089/cell.2013.0081)

Lorca T, Cruzalegui FH, Fesquet D, Cavadore JC, Méry J, Means A & Dorée M 1993 Calmodulin-dependent protein kinase II mediates inactivation of MPF and CSF upon fertilization of Xenopus eggs. *Nature* **366** 270–273. (https://doi.org/10.1038/366270a0)

Lu Y 2015 Are TrpV3 channels present in human oocytes to mediate strontium-induced artificial activation?, p. i236: Abstract book of the 31st ESHRE Annual Meeting, Lisbon, Portugal.

Lu Q, Chen ZJ, Gao X, Ma SY, Li M, Hu JM & Li Y 2006 Oocyte activation with calcium ionophore A23187 and puromycin on human oocytes that fail to fertilize after intracytoplasmic sperm injection. *Zhonghua Fu Chan Ke Za Zhi* **41** 182–185.

Machaca K & Haun S 2000 Store-operated calcium entry inactivates at the germinal vesicle breakdown stage of Xenopus meiosis. *Journal of Biological Chemistry* **275** 38710–38715. (https://doi.org/10.1074/jbc.M007887200)

Machaty Z 2016 Signal transduction in mammalian oocytes during fertilization. *Cell and Tissue Research* **363** 169–183. (https://doi.org/10.1007/s00441-015-2291-8)

Macháty Z, Wang WH, Day BN & Prather RS 1997 Complete activation of porcine oocytes induced by the sulfhydryl reagent, thimerosal. *Biology of Reproduction* **57** 1123–1127.

Madgwick S, Levasseur M & Jones KT 2005 Calmodulin-dependent protein kinase II, and not protein kinase C, is sufficient for triggering cell-cycle resumption in mammalian eggs. *Journal of Cell Science* **118** 3849–3859. (https://doi.org/10.1242/jcs.02506)

Malcuit C, Knott JG, He C, Wainwright T, Parys JB, Robl JM & Fissore RA 2005 Fertilization and inositol 1,4,5-trisphosphate (IP3)-induced calcium release in type-1 inositol 1,4,5-trisphosphate receptor down-regulated bovine eggs. *Biology of Reproduction* **73** 2–13. (https://doi.org/10.1095/biolreprod.104.037333)

Markoulaki S, Matson S, Abbott AL & Ducibella T 2003 Oscillatory CaMKII activity in mouse egg activation. *Developmental Biology* **258** 464–474. (https://doi.org/10.1016/S0012-1606(03)00133-7)

Matthew A 2009 Presynaptic strontium dynamics and synaptic transmission. *Biophysical Journal* **76** 2029–2042.

McLay DW & Clarke HJ 2003 Remodelling the paternal chromatin at fertilization in mammals. *Reproduction* **125** 625–633. (https://doi.org/10.1530/rep.0.1250625)

Mehlmann LM & Kline D 1994 Regulation of intracellular calcium in the mouse egg: calcium release in response to sperm or inositol trisphosphate is enhanced after meiotic maturation. *Biology of Reproduction* **51** 1088–1098. (https://doi.org/10.1095/biolreprod51.6.1088)

Mehlmann LM, Mikoshiba K & Kline D 1996 Redistribution and increase in cortical inositol 1,4,5-trisphosphate receptors after meiotic maturation of the mouse oocyte. *Developmental Biology* **180** 489–498. (https://doi.org/10.1006/dbio.1996.0322)

Miao YL & Williams CJ 2012 Calcium signaling in mammalian egg activation and embryo development: the influence of subcellular localization. *Molecular Reproduction and Development* **79** 742–756. (https://doi.org/10.1002/mrd.22078)

Miao YL, Stein P, Jefferson WN, Padilla-Banks E & Williams CJ 2012 Calcium influx-mediated signaling is required for complete mouse egg activation. *PNAS* **109** 4169–4174. (https://doi.org/10.1073/pnas.1112333109)

Miller N, Biron-Shental T, Sukenik-Halevy R, Klement AH, Sharony R & Berkovitz A 2016 Oocyte activation by calcium ionophore and congenital birth defects: a retrospective cohort study. *Fertility and Sterility* **106** 590.e592–596.e592. (https://doi.org/10.1016/j.fertnstert.2016.04.025)

Miyazaki S 1993 IP3 receptor-mediated spatial and temporal Ca2+ signaling of the cell. *Japanese Journal of Physiology* **43** 409–434. (https://doi.org/10.2170/jjphysiol.43.409)

Miyazaki S, Yuzaki M, Nakada K, Shirakawa H, Nakanishi S, Nakade S & Mikoshiba K 1992 Block of Ca2+ wave and Ca2+ oscillation by antibody to the inositol 1,4,5-trisphosphate receptor in fertilized hamster eggs. *Science* **257** 251–255. (https://doi.org/10.1126/science.1321497)

Moses RM & Kline D 1995 Release of mouse eggs from metaphase arrest by protein synthesis inhibition in the absence of a calcium signal or microtubule assembly. *Molecular Reproduction and Development* **41** 264–273. (https://doi.org/10.1002/mrd.1080410218)

Neupane J, Vandewoestyne M, Ghimire S, Lu Y, Qian C, Van Coster R, Gerris J, Deroo T, Deforce D, De Sutter P *et al*. 2014 Assessment of nuclear transfer techniques to prevent the transmission of heritable mitochondrial disorders without compromising embryonic development competence in mice. *Mitochondrion* **18** 27–33. (https://doi.org/10.1016/j.mito.2014.09.003)

Nikiforaki D, Vanden Meerschaut F, De Gheselle S, Qian C, Van den Abbeel E, De Vos WH, Deroo T, De Sutter P & Heindryckx B 2014*a* Sperm involved in recurrent partial hydatidiform moles cannot induce the normal pattern of calcium oscillations. *Fertility and Sterility* **102** 581.e581–588.e581. (https://doi.org/10.1016/j.fertnstert.2014.05.004)

Nikiforaki D, Vanden Meerschaut F, Qian C, De Croo I, Lu Y, Deroo T, Van den Abbeel E, Heindryckx B & De Sutter P 2014*b* Oocyte cryopreservation and in vitro culture affect calcium signalling during human fertilization. *Human Reproduction* **29** 29–40. (https://doi.org/10.1093/humrep/det404)

Nikiforaki D, Vanden Meerschaut F, de Roo C, Lu Y, Ferrer-Buitrago M, de Sutter P & Heindryckx B 2016 Effect of two assisted oocyte activation protocols used to overcome fertilization failure on the activation potential and calcium releasing pattern. *Fertility and Sterility* **105** 798.e792–806.e792. (https://doi.org/10.1016/j.fertnstert.2015.11.007)

Nomikos M, Yu Y, Elgmati K, Theodoridou M, Campbell K, Vassilakopoulou V, Zikos C, Livaniou E, Amso N, Nounesis G *et al*. 2013 Phospholipase Cζ rescues failed oocyte activation in a prototype of male factor infertility. *Fertility and Sterility* **99** 76–85. (https://doi.org/10.1016/j.fertnstert.2012.08.035)

Nomikos M, Sanders JR, Theodoridou M, Kashir J, Matthews E, Nounesis G, Lai FA & Swann K 2014*a* Sperm-specific post-acrosomal WW-domain binding protein (PAWP) does not cause Ca2+ release in mouse oocytes. *Molecular Human Reproduction* **20** 938–947. (https://doi.org/10.1093/molehr/gau056)

Nomikos M, Theodoridou M, Elgmati K, Parthimos D, Calver BL, Buntwal L, Nounesis G, Swann K & Lai FA 2014*b* Human PLCζ exhibits superior fertilization potency over mouse PLCζ in triggering the Ca2+ oscillations required for mammalian oocyte activation. *Molecular Human Reproduction* **20** 489–498. (https://doi.org/10.1093/molehr/gau011)

Nuccitelli R, Yim DL & Smart T 1993 The sperm-induced Ca2+ wave following fertilization of the Xenopus egg requires the production of Ins(1,4,5)P3. *Developmental Biology* **158** 200–212. (https://doi.org/10.1006/dbio.1993.1179)

Ozil JP 1998 Role of calcium oscillations in mammalian egg activation: experimental approach. *Biophysical Chemistry* **72** 141–152. (https://doi.org/10.1016/S0301-4622(98)00130-6)

Ozil JP, Markoulaki S, Toth S, Matson S, Banrezes B, Knott JG, Schultz RM, Huneau D & Ducibella T 2005 Egg activation events are regulated by the duration of a sustained [Ca2+]cyt signal in the mouse. *Developmental Biology* **282** 39–54. (https://doi.org/10.1016/j.ydbio.2005.02.035)

Ozil JP, Banrezes B, Tóth S, Pan H & Schultz RM 2006 Ca2+ oscillatory pattern in fertilized mouse eggs affects gene expression and development to term. *Developmental Biology* **300** 534–544. (https://doi.org/10.1016/j.ydbio.2006.08.041)

Parrington J, Brind S, De Smedt H, Gangeswaran R, Lai FA, Wojcikiewicz R & Carroll J 1998 Expression of inositol 1,4,5-trisphosphate receptors in mouse oocytes and early embryos: the type I isoform is upregulated in oocytes and downregulated after fertilization. *Developmental Biology* **203** 451–461. (https://doi.org/10.1006/dbio.1998.9071)

Payne C & Schatten G 2003 Golgi dynamics during meiosis are distinct from mitosis and are coupled to endoplasmic reticulum dynamics until fertilization. *Developmental Biology* **264** 50–63. (https://doi.org/10.1016/j.ydbio.2003.08.004)

Perocchi F, Gohil VM, Girgis HS, Bao XR, McCombs JE, Palmer AE & Mootha VK 2010 MICU1 encodes a mitochondrial EF hand protein required for Ca(2+) uptake. *Nature* **467** 291–296. (https://doi.org/10.1038/nature09358)

Perry AC & Verlhac MH 2008 Second meiotic arrest and exit in frogs and mice. *EMBO Reports* **9** 246–251. (https://doi.org/10.1038/embor.2008.22)

Phillips KP, Petrunewich MA, Collins JL, Booth RA, Liu XJ & Baltz JM 2002 Inhibition of MEK or cdc2 kinase parthenogenetically activates mouse eggs and yields the same phenotypes as Mos(-/-) parthenogenotes. *Developmental Biology* **247** 210–223. (https://doi.org/10.1006/dbio.2002.0680)

Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA & Pozzan T 1998 Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca2+ responses. *Science* **280** 1763–1766. (https://doi.org/10.1126/science.280.5370.1763)

Rogers NT, Hobson E, Pickering S, Lai FA, Braude P & Swann K 2004 Phospholipase Czeta causes Ca2+ oscillations and parthenogenetic activation of human oocytes. *Reproduction* **128** 697–702. (https://doi.org/10.1530/rep.1.00484)

Rogers NT, Halet G, Piao Y, Carroll J, Ko MS & Swann K 2006 The absence of a Ca(2+) signal during mouse egg activation can affect parthenogenetic preimplantation development, gene expression patterns, and blastocyst quality. *Reproduction* **132** 45–57. (https://doi.org/10.1530/rep.1.01059)

Runft LL, Watras J & Jaffe LA 1999 Calcium release at fertilization of Xenopus eggs requires type I IP(3) receptors, but not SH2 domain-mediated activation of PLCgamma or G(q)-mediated activation of PLCbeta. *Developmental Biology* **214** 399–411. (https://doi.org/10.1006/dbio.1999.9415)

Runft LL, Jaffe LA & Mehlmann LM 2002 Egg activation at fertilization: where it all begins. *Developmental Biology* **245** 237–254. (https://doi.org/10.1006/dbio.2002.0600)

Rybouchkin A, Dozortsev D, de Sutter P, Qian C & Dhont M 1995 Intracytoplasmic injection of human spermatozoa into mouse oocytes: a useful model to investigate the oocyte-activating capacity and the karyotype of human spermatozoa. *Human Reproduction* **10** 1130–1135. (https://doi.org/10.1093/oxfordjournals.humrep.a136105)

Rybouchkin A, Dozortsev D, de Sutter PD & Dhont M 1996 Factors affecting the role of the spindle during early response of mouse oocytes to ethanol stimulation. *Journal of Experimental Zoology* **275** 469–475. (https://doi.org/10.1002/(SICI)1097-010X(19960815)275:6<469::AID-JEZ9>3.0.CO;2-M)

Santella L & Dale B 2015 Assisted yes, but where do we draw the line? *Reproductive BioMedicine Online* **31** 476–478. (https://doi.org/10.1016/j.rbmo.2015.06.013)

Sanusi R, Yu Y, Nomikos M, Lai FA & Swann K 2015 Rescue of failed oocyte activation after ICSI in a mouse model of male factor infertility by recombinant phospholipase Cζ. *Molecular Human Reproduction* **21** 783–791. (https://doi.org/10.1093/molehr/gav042)

Saunders CM, Larman MG, Parrington J, Cox LJ, Royse J, Blayney LM, Swann K & Lai FA 2002 PLC zeta: a sperm-specific trigger of Ca(2+) oscillations in eggs and embryo development. *Development* **129** 3533–3544.

Shoji S, Yoshida N, Amanai M, Ohgishi M, Fukui T, Fujimoto S, Nakano Y, Kajikawa E & Perry AC 2006 Mammalian Emi2 mediates cytostatic arrest and transduces the signal for meiotic exit via Cdc20. *EMBO Journal* **25** 834–845. (https://doi.org/10.1038/sj.emboj.7600953)

Shuai JW, Yang DP, Pearson JE & Rudiger S 2009 An investigation of models of the IP3R channel in Xenopus oocyte. *Chaos* **19** 037105. (https://doi.org/10.1063/1.3156402)

Smedler E & Uhlén P 2014 Frequency decoding of calcium oscillations. *Biochimica et Biophysica Acta* **1840** 964–969. (https://doi.org/10.1016/j.bbagen.2013.11.015)

Stathopulos PB, Li GY, Plevin MJ, Ames JB & Ikura M 2006 Stored Ca2+ depletion-induced oligomerization of stromal interaction molecule 1 (STIM1) via the EF-SAM region: an initiation mechanism for capacitive Ca2+ entry. *Journal of Biological Chemistry* **281** 35855–35862. (https://doi.org/10.1074/jbc.M608247200)

Stricker SA 1999 Comparative biology of calcium signaling during fertilization and egg activation in animals. *Developmental Biology* **211** 157–176. (https://doi.org/10.1006/dbio.1999.9340)

Sun L, Haun S, Jones RC, Edmondson RD & Machaca K 2009 Kinase-dependent regulation of inositol 1,4,5-trisphosphate-dependent

Ca2+ release during oocyte maturation. *Journal of Biological Chemistry* **284** 20184–20196. (https://doi.org/10.1074/jbc.M109.004515)

Suzuki T, Yoshida N, Suzuki E, Okuda E & Perry AC 2010 Full-term mouse development by abolishing Zn2+-dependent metaphase II arrest without Ca2+ release. *Development* **137** 2659–2669. (https://doi.org/10.1242/dev.049791)

Swann K 1991 Thimerosal causes calcium oscillations and sensitizes calcium-induced calcium release in unfertilized hamster eggs. *FEBS Letters* **278** 175–178. (https://doi.org/10.1016/0014-5793(91)80110-O)

Swann K & Lai FA 2013 PLCζ and the initiation of Ca(2+) oscillations in fertilizing mammalian eggs. *Cell Calcium* **53** 55–62. (https://doi.org/10.1016/j.ceca.2012.11.001)

Swann K & Lai FA 2016 Egg activation at fertilization by a soluble sperm protein. *Physiological Reviews* **96** 127–149. (https://doi.org/10.1152/physrev.00012.2015)

Takahashi T, Kikuchi T, Kidokoro Y & Shirakawa H 2013 Ca(2)(+) influx-dependent refilling of intracellular Ca(2)(+) stores determines the frequency of Ca(2)(+) oscillations in fertilized mouse eggs. *Biochemical and Biophysical Research Communications* **430** 60–65. (https://doi.org/10.1016/j.bbrc.2012.11.024)

Tatone C, Delle Monache S, Iorio R, Caserta D, Di Cola M & Colonna R 2002 Possible role for Ca(2+) calmodulin-dependent protein kinase II as an effector of the fertilization Ca(2+) signal in mouse oocyte activation. *Molecular Human Reproduction* **8** 750–757. (https://doi.org/10.1093/molehr/8.8.750)

Tesarik J & Sousa M 1995 More than 90% fertilization rates after intracytoplasmic sperm injection and artificial induction of oocyte activation with calcium ionophore. *Fertility and Sterility* **63** 343–349. (https://doi.org/10.1016/S0015-0282(16)57366-X)

Tesarik J, Rienzi L, Ubaldi F, Mendoza C & Greco E 2002 Use of a modified intracytoplasmic sperm injection technique to overcome sperm-borne and oocyte-borne oocyte activation failures. *Fertility and Sterility* **78** 619–624. (https://doi.org/10.1016/S0015-0282(02)03291-0)

Tóth S, Huneau D, Banrezes B & Ozil JP 2006 Egg activation is the result of calcium signal summation in the mouse. *Reproduction* **131** 27–34. (https://doi.org/10.1530/rep.1.00764)

Tripathi A, Kumar KV & Chaube SK 2010 Meiotic cell cycle arrest in mammalian oocytes. *Journal of Cellular Physiology* **223** 592–600. (https://doi.org/10.1002/jcp.22108)

Uranga JA, Pedersen RA & Arechaga J 1996 Parthenogenetic activation of mouse oocytes using calcium ionophores and protein kinase C stimulators. *International Journal of Developmental Biology* **40** 515–519.

van Blerkom J, Cohen J & Johnson M 2015 A plea for caution and more research in the 'experimental' use of ionophores in ICSI. *Reproductive BioMedicine Online* **30** 323–324. (https://doi.org/10.1016/j.rbmo.2015.02.002)

Vanden Meerschaut F, Nikiforaki D, De Gheselle S, Dullaerts V, Van den Abbeel E, Gerris J, Heindryckx B & De Sutter P 2012 Assisted oocyte activation is not beneficial for all patients with a suspected oocyte-related activation deficiency. *Human Reproduction* **27** 1977–1984. (https://doi.org/10.1093/humrep/des097)

Vanden Meerschaut F, Leybaert L, Nikiforaki D, Qian C, Heindryckx B & De Sutter P 2013 Diagnostic and prognostic value of calcium oscillatory pattern analysis for patients with ICSI fertilization failure. *Human Reproduction* **28** 87–98. (https://doi.org/10.1093/humrep/des368)

Vanden Meerschaut F, D'Haeseleer E, Gysels H, Thienpont Y, Dewitte G, Heindryckx B, Oostra A, Roeyers H, Van Lierde K & De Sutter P 2014*a* Neonatal and neurodevelopmental outcome of children aged 3–10 years born following assisted oocyte activation. *Reproductive BioMedicine Online* **28** 54–63. (https://doi.org/10.1016/j.rbmo.2013.07.013)

Vanden Meerschaut F, Nikiforaki D, Heindryckx B & De Sutter P 2014*b* Assisted oocyte activation following ICSI fertilization failure. *Reproductive BioMedicine Online* **28** 560–571. (https://doi.org/10.1016/j.rbmo.2014.01.008)

Vanderheyden V, Devogelaere B, Missiaen L, De Smedt H, Bultynck G & Parys JB 2009*a* Regulation of inositol 1,4,5-trisphosphate-induced Ca2+ release by reversible phosphorylation and dephosphorylation. *Biochimica et Biophysica Acta* **1793** 959–970. (https://doi.org/10.1016/j.bbamcr.2008.12.003)

Vanderheyden V, Wakai T, Bultynck G, De Smedt H, Parys JB & Fissore RA 2009*b* Regulation of inositol 1,4,5-trisphosphate receptor type 1 function during oocyte maturation by MPM-2 phosphorylation. *Cell Calcium* **46** 56–64. (https://doi.org/10.1016/j.ceca.2009.04.004)

Vasilev F, Chun JT, Gragnaniello G, Garante E & Santella L 2012 Effects of ionomycin on egg activation and early development in starfish. *PLoS ONE* **7** e39231. (https://doi.org/10.1371/journal.pone.0039231)

Versieren K, Heindryckx B, Lierman S, Gerris J & De Sutter P 2010 Developmental competence of parthenogenetic mouse and human embryos after chemical or electrical activation. *Reproductive BioMedicine Online* **21** 769–775. (https://doi.org/10.1016/j.rbmo.2010.07.001)

Wakai T, Vanderheyden V, Yoon SY, Cheon B, Zhang N, Parys JB & Fissore RA 2012 Regulation of inositol 1,4,5-trisphosphate receptor function during mouse oocyte maturation. *Journal of Cellular Physiology* **227** 705–717. (https://doi.org/10.1002/jcp.22778)

Wakai T, Zhang N, Vangheluwe P & Fissore RA 2013 Regulation of endoplasmic reticulum Ca2+ oscillations in mammalian eggs. *Journal of Cell Science* **141** e207. (https://doi.org/10.1242/jcs.136549)

Winston N, Johnson M, Pickering S & Braude P 1991 Parthenogenetic activation and development of fresh and aged human oocytes. *Fertility and Sterility* **56** 904–912. (https://doi.org/10.1016/S0015-0282(16)54663-9)

Yanagida K, Morozumi K, Katayose H, Hayashi S & Sato A 2006 Successful pregnancy after ICSI with strontium oocyte activation in low rates of fertilization. *Reproductive BioMedicine Online* **13** 801–806. (https://doi.org/10.1016/S1472-6483(10)61027-9)

Yeste M, Jones C, Amdani SN, Patel S & Coward K 2016 Oocyte activation deficiency: a role for an oocyte contribution? *Human Reproduction Update* **22** 23–47. (https://doi.org/10.1093/humupd/dmv040)

Yu Y, Saunders CM, Lai FA & Swann K 2008 Preimplantation development of mouse oocytes activated by different levels of human phospholipase C zeta. *Human Reproduction* **23** 365–373. (https://doi.org/10.1093/humrep/dem350)

Yu Y, Nomikos M, Theodoridou M, Nounesis G, Lai FA & Swann K 2012 PLCζ causes Ca(2+) oscillations in mouse eggs by targeting intracellular and not plasma membrane PI(4,5)P(2). *Molecular Biology of the Cell* **23** 371–380. (https://doi.org/10.1091/mbc.E11-08-0687)

Zhang N, Yoon SY, Parys JB & Fissore RA 2015 Effect of M-phase kinase phosphorylations on type 1 inositol 1,4,5-trisphosphate receptor-mediated Ca2+ responses in mouse eggs. *Cell Calcium* **58** 476–488. (https://doi.org/10.1016/j.ceca.2015.07.004)

Received 20 February 2017
First decision 13 March 2017
Revised manuscript received 6 November 2017
Accepted 9 November 2017