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Review article

Oocyte activation: The impact of calcium signals on fertilization

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Abstract

Fertilization is triggered by changes in intracellular calcium concentration. In mammals, these transients in ooplasmic calcium concentration take the form of repetitive spikes, so called calcium oscillations (Ca^{2+}-oscillations). These oscillations are important for relieve of meiotic arrest and to induce all the other events of oocyte activation. Although a surface mediated way of oocyte activation has been proposed, there is now substantial evidence to suggest that the sperm cell induces these Ca^{2+}-oscillations by introducing a sperm specific phospholipase C, PLCζ, in the ooplasm. Ca^{2+}-oscillations are also observed after intracytoplasmic sperm injection (ICSI), a successful technique in human assisted reproduction. In the rare cases that no fertilization is observed following ICSI, this may be due to a deficiency in PLCζ. However, artificial activating the oocytes after ICSI by increasing the calcium concentration can restore fertilization rates in these cases and support further development, as evidenced by successful pregnancies. Further evaluation of the current protocols for assisted oocyte activation is appropriate and investigation of the future application of PLCζ is warranted.

Key words: *Calcium, Oocyte activation, PLCζ, ICSI.*

Introduction

Calcium is a universal secondary messenger in cells controlling diverse biological processes such as proliferation, differentiation, axis formation, transcriptional activation and apoptosis (1). It plays a major role at fertilization and is thus already involved at the very beginning of life. Sperm not only delivers its genetic material but also triggers rises in intracellular calcium concentration ($[Ca^{2+}]_i$) and consequently awakens the oocyte which is blocked at the metaphase of the second meiotic division (MII arrest). For all species an increase in $[Ca^{2+}]_i$ is necessary and sufficient for the completion of oocyte activation and initiation of embryonic development (2,3). In mammalian oocytes, a series of spikes in $[Ca^{2+}]_i$ or so called Ca^{2+}-oscillations, are triggered upon sperm entry. These Ca^{2+}- oscillations are needed to relieve MII arrest and to provoke all the other events of oocyte activation, such as the cortical reaction , maternal mRNA recruitment, pronuclear development and mitotic cleavage (4). MII arrest is characterized by a high level of the cyclinB/cdk1 complex, also known as maturation promoting factor (MPF). MPF prompts metaphase II and is thought to be involved in several central features of cell division, such as disassembly of the nucleus, chromosome condensation, cytoskeletal rearrangements and arrest in transcriptional activity (5).

MPF activity in the MII-arrested oocyte is regulated by the activity of a cytostatic factor (CSF). During MII arrest, CSF indeed prevents the destruction of MPF by keeping the anaphase-promoting complex (APC) inactive (figure 1). When active, the latter complex labels cyclin B for degradation through the ubiquitin pathway. At fertilization, MPF levels must decrease sufficiently to enable the oocyte to complete the meiosis and initiate oocyte activation (6).

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Several theories on how the sperm sparks Ca^{2+}-oscillations and thus activates the oocyte have been proposed. In the surface-mediated model of oocyte activation, binding of a sperm protein on a putative oocyte receptor would activate PLC which in turn would produce inositol 1,4,5 trisphosphate, which induces release of Ca^{2+} from intracellular stores and subsequently triggers Ca^{2+}-oscillations in the oocyte (2). However, although in echinoderms and amphibians there is evidence of involvement of such an oocyte PLC, no such evidence has been forthcoming for mammals and in general there is a lack of evidence showing that a surface protein that mediates sperm-oocyte binding is linked with intracellular signalling leading to oocyte activation (7). A second model in which a soluble sperm factor introduced into the ooplasm at gamete fusion activates the oocyte was proposed by Dale et al, on the basis that microinjection of sea urchin sperm extract triggered the cortical reaction in sea urchin eggs (8). Subsequently Swann and others showed that injection of mammalian sperm extracts into eggs and oocytes resulted in a pattern of Ca^{2+} release similar to that seen at fertilization and also triggered egg activation (9, 10).

The oocyte activating factor PLCζ

The identity of the sperm factor remained unknown for some time, but then a sperm specific phospholipase C named PLCζ was identified which had the expected properties of the oocyte activating factor (11). Injection of recombinant mouse and human PLCζ RNA into mouse oocytes not only evokes Ca^{2+}-oscillations similar to those induced by sperm but also promotes subsequent embryo development (11,12).

A similar response was obtained by microinjecting recombinant mouse PLCζ protein into mouse oocytes (13). Immunolocalization studies have shown that the pattern of localization of PLCζ in sperm of both rodents and humans is in the expected location for an oocyte activation factor, namely the perinuclear theca (14,15). Moreover, sperm from transgenic mice expressing short hairpin RNAs targeting PLCζ exhibited reduced amounts of PLCζ, and when injected into mouse oocytes, induced Ca^{2+} oscillations that ended prematurely (16).

PLCζ catalyzes the formation of inositol trisphosphate, which triggers the release of Ca^{2+} from intracellular stores and subsequently induces the calcium oscillations in the oocyte (2). Calcium will activate calmodulin dependent kinase II (CaMKII), an enzyme known to be sensitive to the frequency of calcium oscillations (17). CaMKII directly activates APC and also promotes the degradation of CSF (18).

As a result, the APC is liberated from its repression by CSF and the inhibition of MPF will be relieved. APC activation causes a decline in MPF activity and the concomitant release from the MII arrest (Figure 1).

The Ca^{2+}-oscillations prevent the MPF levels to return to active levels and bring about successful activation (4).

Oocyte activation in human assisted reproduction technology

Calcium oscillations are also observed after intracytoplasmic sperm injection (ICSI), a highly successful technique used in human assisted reproductive medicine in case of male infertility (19). Only in a small percentage of the patients failed fertilization is observed after ICSI (20). A specific group of patients who often face failed fertilization are men with globozoospermia, a rare disorder characterized by round-headed, acrosome-less sperm cells (21). The lack of an acrosome was considered to be the cause of infertility in these patients (22).

The introduction of ICSI led to several successful attempts resulting in pregnancies following ICSI with globozoospermia sperm, however fertilization rates were low (23). Moreover, there have been several reports of unsuccessful ICSI attempts in cases of globozoospermia (24).

This suggests that besides the inability to interact with the female gamete due to an abnormal acrosome, globozoospermia sperm may have additional defects that affect their fertilization capability. One study employing the mouse oocyte activation test (MOAT) to analyse spermatozoa from globozoospermic men indicated that they could not successfully activate oocytes (25).

Currently the presence of PLCζ in sperm cells is under investigation and preliminary data confirm that sperm from globozoospermic men contain lower amounts of PLCζ as compared to normal (26). In addition, also other cases of failed fertilization may be explained by defects in the PLCζ protein.

Assisted oocyte activation

Before considering gamete donation, artificial activation of the oocytes may be proposed as an option to couples who experienced repeated cycles with failed fertilization after ICSI. Mammalian oocytes such as murine, rabbit, bovine, rat, porcine and also human oocytes can namely be activated

just by increasing $[Ca^{2+}]_i$ using electrical activation, ethanol treatment or Ca^{2+} ionophore challenge (27-32).

Interestingly, this technique of artificially induced oocyte activation at the time of ICSI or assisted oocyte activation (AOA) by using for example calcium ionophore to increase $[Ca^{2+}]_i$ can restore normal fertilization rates in these cases and support further development as evidenced by successful pregnancies and deliveries reported (33-35). However, some concerns still exist about the use of calcium ionophore as an activating agent in human ART (33,35). And although no adverse effects of ionomycin on in vitro or in vivo mouse embryo development were noticed (36), a negative long-term effect of this compound can not be excluded at present. In addition, some studies have shown that the Ca^{2+}-transients induced during oocyte activation may influence the number of embryos reaching the blastocyst stage and even postnatal development (37,38). Ionomycin induces only a single Ca^{2+} transient in human oocytes (39) and no Ca^{2+}-oscillations are triggered after exposing mouse oocytes injected with human sperm deficient in oocyte activating capacity (40). Successful clinical application in the human, however, suggests that this does not seem to prevent further embryonic development.

In attempt to induce calcium changes in more physiological way, other techniques have been introduced such as a modified ICSI technique (41) and recently also strontium was applied successfully in a clinical setting (42) . Strontium acts like a surrogate of Ca^{2+} in mouse oocytes and causes a series of Ca^{2+} -oscillations similar to those observed at fertilization (28,43). However, the observation that no Ca^{2+}-oscillations were triggered in human oocytes treated with strontium (12) suggests that other mechanisms than described for mouse oocytes are involved (43) and that an activation protocol based on strontium should still be treated with caution. Interestingly, the discovery of the oocyte activating factor PLCζ (11) and the observation of PLCζ deficiencies in infertile men (26), may lead to the development of a more physiological method of triggering oocyte activation in case of sperm borne fertilization failure after ICSI, for example by co-injecting a recombinant PLCζ at the time of ICSI.

Unraveling the mechanism of oocyte activation and determining the role of calcium in this process has resulted in promising applications in human ART. However, follow-up of the children born after assisted oocyte activation remains necessary, whatever protocol is used.

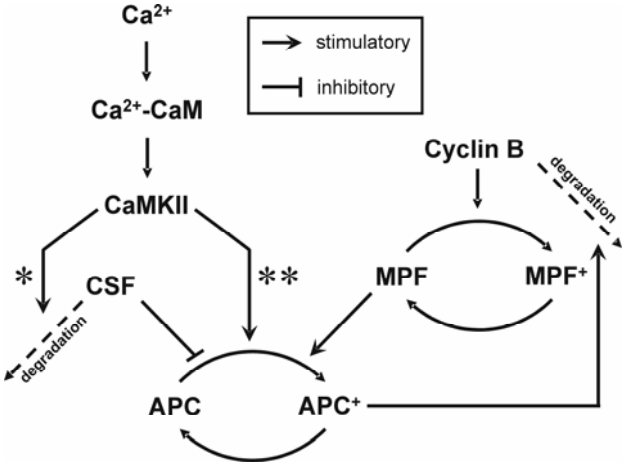

Figure 1: Schematic diagram illustrating the calcium-induced relief from metaphase II arrest and cell cycle resumption. The Ca^{2+}-CaM – CaMKII axis directly and thus rapidly activates APC (**) and also promotes the degradation of CSF, a process with slower kinetics (*). CSF degradation releases the inhibition of APC. Activated APC (APC^+) promotes the degradation of Cyclin B and as a consequence, results in MPF deactivation. This culminates in the abolishment of MII arrest and resumption of the cell cycle. (With kind permission of Leybaert L and Dupont G).

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