Research Article

The role of Ca^{+2} oscillator in oocytes activation with different types of sperms

Aya Al-Ibraheemi[1], Noureddine Louanjli[2], Mohamed Zarqaoui[3], Mohamed Ennaji[4], Wassym R. Senhaji[5], Mustafa Zakaria[6*], Ritu S. Santwani[7] and Romaissa Boutiche[8]

[1]Sustainary Health, Art Irifiv Scientific Research Group, England, UK
[2,3]ART IRIFIV Scientific Research Group, Casablanca, Morocco
[4,5,6]IRIFIV Fertility Center, Casablanca, Morocco
[7]Department of Obstetrics and Gynaecology, VIMS Medical College, Garjula, India
[8]Laboratory IVF Algeria, Rotaby Fertility Center, Algiers, Algiera

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ABSTRACT

This research article has been made to demonstrate the oocytes activation process and how it's related to Ca2+ oscillation. Moreover, the research shows how Ca2+ oscillation is transition pattern can be affected by the sperm types. It has been found that the oocysts activation depends on intercellular factors and single transduction which is mainly activated by Ca2+ oscillation. Ca2+ oscillation induction can have a different pattern in different spices, but it depends chiefly on one factor which the sperm-oocytes fusion. Through fertilization, the spermatozoon produces a series of low- long-lasting Ca2+ oscillations frequency. It is usually believed that these oscillations are caused by to Ca2+ release into the Inositol 1,4,5-Trisphosphate (InsP3) receptor. Thus, the Ca2+ oscillation can also relay on the type of sperm injected to the oocytes where it has been found that round spermatid is not as effective as spermatozoon to induce Ca2+ oscillation. Although round spermatid can be used in cases such as severe azoospermia, where no spermatozoon is available, with the help of artificial oocytes activation.

Keywords: Oocyte activation • Calcium oscillations • Sperm types • Artificial oocytes activation • What causes spermatozoa to produce a Ca2+ influx?

Abbreviations: ZP: Zona Pellucida; ICSI: Intracytoplasmic Sperm Injection; SF: Sperm Factor; MPF: Maturation Promoting Factor; PLCζ: Phospholipase C Isoform Zeta; AOA: Artificial Oocyte Activation

Introduction

What is oocytes activation?

Oocyte activation is a set of processes which start in the oocyte when a sperm evades the oolemma throughout fertilization. The calcium release process begins when the sperm entry induces calcium release into the oocyte. The fertilization stops metaphase-II development and converts the oocyte into a fertilized egg ready to start embryo development (embryogenesis). Oocytes activation is an event that can happen in-vivo and in-vitro whenever a sperm fertilizes an oocyte successfully, with a tightly regulated step [1]. The steps start with Zona Pellucida (ZP) penetrating by the sperm, where it then encounters physical and chemical alterations by the extrusion of the cortical granules from the oocyte to block polyspermy entry and to protect and maintain the health embryo development. In ZP binding, several activation factors are needed, such as ZP1, 2, 3 glycoproteins, ZP 1 and 2 are structural glycoproteins while ZP3 is sperm binding glycoproteins (Figure 1).

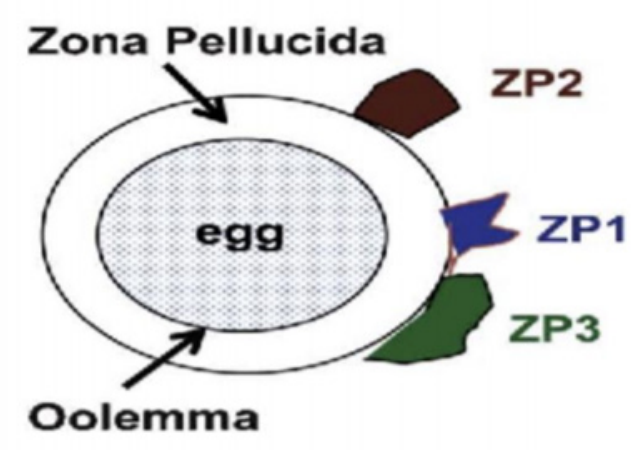

Figure 1: Oocytes zone pellucida glycoproteins. The image above illustrates the sperm binding sites in the oolemma membrane, and the Zona binding region.

Corresponding Author:
Mustafa Zakaria, IRIFIV Fertility Center, Casablanca, Morocco, Telephone: +467 01943817
E-mail: dr.mustafazakaria@gmail.com

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After ZP drilling, fertilisation fusion starts where spermatozoa enter the perivitelline space, through exposing the equatorial sperm surface parallel to the oolemma. Fertilisation fusion enables the fusion of the plasma membranes of both gametes (Figure 2).

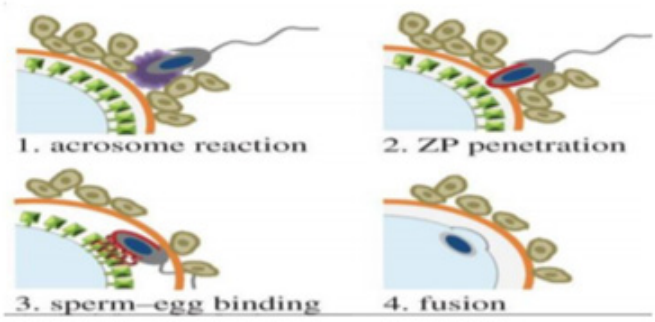

Figure 2: Mammalian fertilization member fusion. The image above demonstrates the sperm fusion, where sperm is engulfed, and induce cortical reaction.

Oocytes activation describes a cellular transformation from an extremely differentiated oocyte into a totipotent embryo cell. The critical elements of this transition activation is a single initiating signal at the start of this transformation, that assures that all of the downstream signals and subsequent events are precisely regulated within the cell (this is particularly crucial as each event is controlled at various levels). Through oocytes activation, an increase in cytosolic Ca2 + automatically activates the effector proteins CaMKII and calcineurin, to start this cellular transition. These proteins then have the potential to expand the initial signal by phosphorylation of various target proteins, activating multiple processes within the cell (Figure 3).

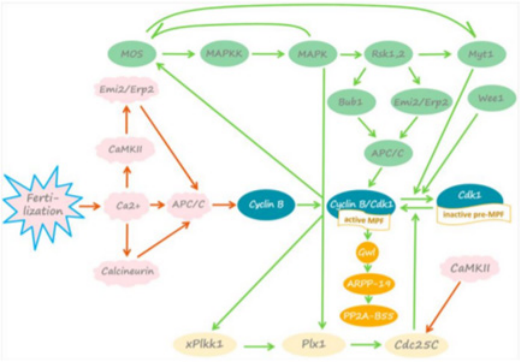

Figure 3: Signaling pathways of fertilized oocytes. After fertilization, a signaling pathway begins with a Ca+2 induction that induces efforts proteins activation.

Calcium oscillations

As illustrated previously, the primary key to start all the cytological alterations in fertilized oocytes is a series of intracellular calcium increases, which begin straightaway after spermatozoon–oocyte fusion. Invertebrates, the release of calcium from intracellular granules within the endoplasmic reticulum is mostly the primary induction of the intracellular calcium rise. Intracellular calcium oscillations after fertilization proceed from a few minutes to around several hours, depending mainly on the species. Gradual drop and eventual termination of calcium oscillations normally happen when pronuclei are formed [2]. In mammals, the Ca2+ after the response of oocytes to the fertilizing by spermatozoon displays a range of repetitive spikes known as Ca2+ oscillation; this can also be induced through Intracytoplasmic Sperm Injection (ICSI) similar to those of regular fertilization. Calcium waves are intermittent rises in the concentration of Ca2+ in a limited spatial manner, such as local areas in the cytoplasm, that develop in a wave-like fashion. It assists as a primary signalling mechanism, both intracellularly and intercellularly. Usually, free Ca2+ concentration in the cytosol is actively sustained at a significantly lower level than extracellular levels; thus, this manner enables cytosol to be a site of notable, rapid change in Ca2+ concentration in response to small amounts of Ca2+ signaling from extracellular medium or intracellular parts [3]. The oscillatory signal is started when the equilibrium of cytosolic Ca2+ concentration is disturbed. This happens when the cell receives stimulatory signals that stimulate channels which transport Ca2+ into the cytoplasm. Such channels are found on the existence of the plasma membrane and endoplasmic/sarcoplasmic reticulum. Moreover, the pattern of calcium influx varies from a species to another and can range from a Ca2+ increase every 2 min, to a Ca2+ raise every one hour. For instance, in mammals, there is a notable and long-lasting influx of Ca2+ oscillations observed in the oocytes (Figure 4).

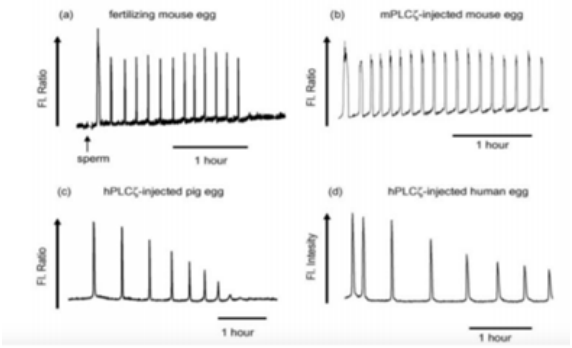

Figure 4: Schematic illustration displaying the Ca2+ influx patterns in different species.

Besides, in Yazawa H, research, four patterns of intracellular calcium influx has been identified after spermatid/sperm-injected oocytes (Types A-D). Type A: typical oscillation pattern includes a repeated spike-shaped Ca2+ rises which take around 2–10 min. Type B: irregular oscillation pattern that has a mixture of continuous Ca2+ rise and oscillation pattern. Type C: quick way, consists of several (1–4) transient Ca2+ rises. Type D: nonresponse model, with no Ca2+ increases through observation periods [3]. The first straightforward measures of intracellular calcium oscillations caused by spermatozoa in human oocytes were completed in the early 1990s. The study showed that calcium oscillations were successfully observed in seven zona-free and three zona-intact oocytes produced with spermatozoa for 4 to 5 h. The differences began 20 to 35 min after the induction of spermatozoa into zona pellucida - free oocytes; the recorded amplitude of oscillations reached up to 2.25 μM initially and declined over time in both zona-free and zona-intact oocytes. The incidence of alteration was observed to vary amongst oocytes but remains constant within individual oocytes.

Most of the researches regarding oocytes activation and Ca2+ oscillations proposed that a factor called Sperm Factor (SF) stim-

ulates Ca2+ oscillations and induce oocytes activation. Two processes have been submitted by which the sperm can start the signalling cascades that end up in Ca2+ oscillations. The first mechanism is that the sperm functions as an oocytes plasma membrane receptor proposed being a G-protein or a tyrosine kinase-coupled receptor, that simultaneous sperm binding initiates the PI pathway, pointing to Ca2+ release. Still, there is no definite proof for a sperm surface receptor on the plasma membrane of an oocyte able of signalling Ca2+ release. The second mechanism is that following gamete fusion; the sperm injects a cytosolic factor into the oocytes that induce (Ca2+) oscillations through interacting with a directly unknown target. On the other hand, in ART, direct injection of a real sperm into the cytosol of mammalian oocytes, that avoid any connection within the sperm and oocytes plasma membranes, stimulates Ca2+ responses that follow those recognized through fertilization. However, sperm-derived proteins such as PLC and several other placed in potentially varying sperm parts have been suggested to be the active components of Sperm Factor (SF). There are several reasons for the importance of calcium oscillation process for oocytes activation. Firstly, Ca-fluctuations are needed to assist second meiotic division (MII) arrest and to stimulate all the other phases of oocyte activation, like the cortical reaction, maternal mRNA recruitment, pronuclear development and mitotic cleavage. MII arrest is maintained through a high level of the cyclin B/cdk1 complex, also recognised as Maturation Promoting Factor (MPF). MPF helps metaphase II and is thought to be required in various central characteristics of cell division, such as disassembly of a particular nucleus, chromosome condensation, cytoskeletal rearrangements and arrest in transcriptional activity. Moreover, It has been stated that in mouse embryos, oocytes activation without Ca2+ oscillation, results in a smaller number of inner mass cells with a higher quantity of apoptotic cells than embryos with Ca2+ fluctuation. Consequently, it has been proposed that the small number of cells per blastocyst in embryos might relate to a lack of Ca2+ oscillation [4].

Calcium oscillations and sperm type

Throughout several and various researches around Ca2+ oscillation, it has been found that the indication of the Ca2+ can also depend on the type of sperms that fertilize the oocytes.

For instance, when immature sperm cells such as round spermatids (is the haploid gamete that results from the division of secondary spermatocytes, which then develop to elongated spermatids) were injected into oocytes, none of them was activated, and no Ca2+ responses were recorded. These findings proved that round spermatids can't activate oocyte, but it cannot induce Ca2+ oscillation. However, when an elongated spermatid was injected without any artificial stimulation for oocytes activation, most of the oocytes were typically activated. Also, they presented transient patterns (type C) of Ca2+ fluctuation, not oscillation patterns (type A). On the other hand, when mature spermatozoa were injected, nearly all oocytes were usually activated and presented regular oscillation patterns (type A). These findings prove that the Ca2+ oscillation patterns of oocytes injected with immature sperm cells convert from a transient pattern (type C) to an oscillation pattern (type A) while maturing to spermatozoa. Besides, induction by round spermatid injection can only activate the oocytes if used with electrical stimulation. Furthermore, researchers have found that both round spermatid and spermatids nuclei, can't produce calcium oscillation; in which the capability of the spermatid-associated oscillator is usually lower than in mature spermatozoa [5]. This has been proved when a round spermatids nuclei, has been injected to oocyte supported by an artificial triggering with calcium ionophore; with a control group, where full mature spermatozoa injected oocytes. The cleaved embryos of isolated spermatid nuclei were 14, while those fertilized with the whole spermatozoa had 30 cleaved embryos.

So, what causes spermatozoa to produce a Ca2+ influx?

It has been stated earlier that the spermatozoa stimulate Ca2+ influx into oocytes by a particular protein named oscillogen in hamsters. New researches have recorded that Phospholipase C Zeta (PLC□), a unique sperm-specific factor, is effective in the induction of Ca2+ oscillation in oocytes after sperm-egg membrane fusion. Accordingly, the sperm protein PLC□ produces the release of in oocytes and is mediated via Inositol 1,4,5-Trisphosphate (InsP3) receptor. Furthermore, even when unfertilized, mature oocytes are injected by InsP3 or its derivatives, oscillation arises due to the unique feedback properties of the InsP3 receptors (Figure 5).

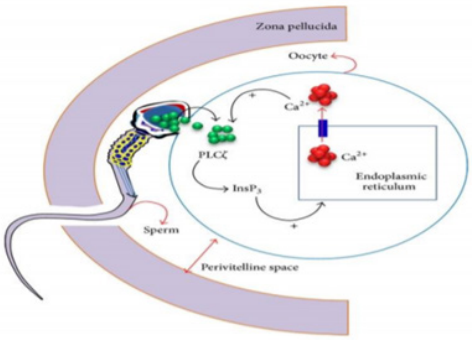

Figure 5: Schematic illustration displaying the Ca2+ influx mechanism in mammalian oocytes. Mature spermatozoa provide the phospholipase C isoform zeta (PLC□) protein after a few minutes of sperm-oocytes fusion. Following the (InsP3) is made as a result of PLC□ hydrolysis that consequently stimulates the nsP3 receptor-mediated Ca2+ release (shown by a red colour circle) from the oocyte endoplasmic reticulum. Concurrently, the increased cytoplasmic Ca2+ leads to Ca2+ positive feedback loop.

There is some suggestion of how human round spermatid injection can be used with effective oocytes activation and successful fertilization. In some cases, the oocyte-activating factor is stimulated after round spermatid freeze-thawing process. However, round spermatid injection alone could produce short Ca2+ oscillations, round spermatid injection with prior electrical stimulation was the most effective way in inducing repetitive, large Ca2+ oscillations [6]. Also, since round spermatids are the tiniest spermatogenic cells in the testis, it can be easily identified from small blood cell leukocytes. In contrast to mice, human spermatids do not present a different chromatin granule, at least with an inverted microscope. It is quite possible that several earlier embryologists, who performed ROSI, indeed picked the wrong cells in that one cannot assume normal development of oocytes injected with spermatogonia or somatic cells [6,7]. The safety of using round spermatid injection is still a concern among researchers and clinicians. Some researchers assume that the inefficacy of round spermatid in-

jection can be a consequence of inadequate male-specific DNA methylation in round spermatids. Still, at least in the mouse, DNA methylation is accomplished in round spermatids and even before meiosis starts. One potential factor may be the incompetent demethylation and remethylation of DNA following the start of spermatid nuclei into mature oocytes. Also, Trichostatin A, an inhibitor of histone deacetylase, that significantly decreases irregular DNA hypermethylation in somatic cell nuclei transplanted into enucleated oocytes, might rise the success rate of human ROSI.

Artificial oocytes activation

Since the first attempt of fertilization using Intracytoplasmic Sperm Injection (ICSI), this method has been broadly applied in treating critical male infertility cases. Moreover, to the use of ejaculated sperm, testicular and epididymal sperm in ICSI, ended in significant levels of fertilization and acceptable pregnancy rates. However, ICSI success rates were considered to be independent of basic sperm parameters; reports have proposed that recurred ICSI failures may be induced by the impact of sperm-derived factors on preimplantation embryo development; which is referred to as paternal effects [8]. As discussed earlier in the article, in mammalian oocytes, sperm plays a role in inducing the rise in internal calcium, as it contains a specific phospholipase C isoform, PLC-zeta, which is produced at an adequate concentration to stimulate calcium spiking in the oocytes. Thus intracellular calcium increase is the signal effective for the meiosis resumption and the beginning of embryo development, consequently performing an essential role during fertilization [9-11]. In azoospermia cases, or where sperms have defected, several pieces of research have shown the benefits of Artificial Oocyte Activation (AOA) with a calcium ionophore to raise the free intracellular calcium, through simulating physiologic cell signalling mechanisms which generally occur in oocyte activation. Different types for artificial oocytes activation have been applied and are usually classified into three subtypes—mechanical, electrical, and chemical stimuli which induce one or several calcium transients. In the mechanical activation of oocytes, oocytes plasma membranes are separated, applying a microneedle to keep calcium osculation, and following a short time, ICSI is performed. Another mechanical technique for oocyte activation is direct calcium microinjection into an oocyte to raise intracellular calcium [12-15].

Conclusion

Electrical stimulation is the second type of AOA which produces calcium influx by the creation of holes in the plasma membrane. The effectiveness of this method depends on hole size, ionic content of the medium, and cell type. Electrical oocyte activation has been successfully applied to bovine and human oocytes. Electrical activation also has been shown to induce reactive oxygen species. Finally, chemical mixtures can likewise produce calcium rise and begin oocyte activation. Chemical oocyte activation has been investigated with the use of composites such as ethanol, calcium ionophore A213187, ionomycin, puromycin, strontium chloride, phorbol ester, and thimerosal. Nevertheless, the effectiveness of these compounds for AOA has been principally restricted to animal models and case reports. However, the efficacy of these compounds for AOA has been chiefly limited to animal models and case reports. These compounds boost the release of intracellular calcium influx, mobilize intracellular calcium by reduction of calcium stores, and promote the influx of extracellular calcium ions. Some of these components can produce a single calcium increase in the oocyte, while other chemicals may induce multiple calcium rises. Though, human oocytes don't efficiently respond to common activators of mammalian oocytes such as the aforementioned chemical components.

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