JRME

Journal of Reproductive Medicine and Embryology

Unveiling the Secrets of Oocyte Activation, Is Calcium the Only Solution?!: A mini-review

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Abstract

Total fertilization failure (TFF) refers to the complete inability of all oocytes in a human IVF cycle to undergo fertilization, even after the administration of intracytoplasmic sperm injection therapy (ICSI). Currently, oocyte activation insufficiency could explain the occurrence of TFF; however, its mechanism remains unclear. Calcium signaling plays a crucial role in oocyte activation events. Following fertilization, oocytes from humans and animals exhibited notable calcium oscillations. Abnormal calcium oscillations following fertilization could be the primary cause of TFF. Examining the literature revealed numerous attempts to investigate the primary cause and discover an effective solution for that alarming occurrence. The most commonly used regimen to overcome oocyte activation deficiency (OAD) is the addition of Calcium, which cannot induce the proper oscillation needed for fertilization. This literature review seeks to address the issue of fertilization failure (FF) by examining the various factors associated with this occurrence and investigating the available techniques for artificial oocyte activation following ICSI.

Keywords: Oocyte activation; Calcium; sperm activation; fertilization failure; ICSI; artificial activation.

Introduction

Oocyte activation deficiency (OAD) is a failure or defect in a coordinated series of events during fertilization, whether sperm or oocyte-borne. It results in the inability of the sperm to undergo activation and complete fertilization, which causes Fertilization Failure (FF) (1).

Mini Review

Total fertilization failure (TFF) refers to the FF of all oocytes in a human in vitro fertilization (IVF) cycle, even after ICSI treatment (2). TFF is poorly understood and is currently attributed to OAD (3).

Oocyte activation events rely on the crucial role of calcium signaling. Calcium oscillations have been observed in both animal and human oocytes following fertilization. The leading cause of TFF may be abnormal calcium oscillations after fertilization (4).

Many studies investigated the molecular mechanisms involved in oocyte activation and FF and the clinical methodologies used to overcome OAD, such as assisted oocyte activation (AOA) (5-7).

Thus far, there is insufficient empirical evidence to establish the effectiveness and safety of such protocols. This current mini-review focuses on the molecular mechanisms of gamete activation before fertilization. It critically evaluates the literature on the involvement of Ca^{+2} oscillations in oocyte activation and the evidence regarding using natural and artificial factors as stimulants for AOA. The discussion will also include AOA's physiological, pathophysiological, and ethical aspects.

Gamete Activation

Gamete activation refers to the transition of both spermatozoa and oocytes from a quiescent state to a developmentally competent state, which is regulated by various cellular and molecular mechanisms (8). The events activating both gametes are interconnected, with shared molecules and signaling pathways. Calcium is crucial in each process stage for both gametes (9).
The successful fertilization process relies on both gametes establishing full competency following the intricate sequence of events required for their activation (10).

I. Sperm Activation

Before the male gamete can commence the necessary steps for effectively fertilizing an oocyte, the spermatozoon must undergo activation, which encompasses various behavioral, physiological, and structural changes (11). Specific changes occur due to exposure to environmental signals, while others arise during the interaction between the spermatozoon and the oocyte and its extracellular investments (12).

The steps include;

1. Changes in Motility

Spermatozoa remain in the testis in a quiescent state and only acquire full motility following ejaculation and capacitation. Intracellular ions play a crucial role in regulating sperm motility, which is closely linked to changes in the membrane potential. Specifically, this process is associated with a hyperpolarization induced by potassium (13).

2. Capacitation and Hyperactivation

Capacitation is a sequence of modifications that provide sperms with the ability to attach to and penetrate the oocyte. These changes encompass increased membrane fluidity, cholesterol efflux, ion fluxes that alter sperm membrane potential, increased tyrosine phosphorylation of proteins, induction of hyperactive motility, and the acrosome reaction (14).

Hyperactivation is characterized by a change in flagellar beating and an increase in the amplitude of flagellar bending. Many enzymes and factors from the female tract, such as arylsulfatase, fucosidase, and taurine, have been implicated in causing capacitation. Moreover, follicular fluid can promote capacitation in vitro (15). A low molecular weight motility factor present in follicular fluid, ovary, uterus, and oviduct can enhance sperm metabolism and movement by reducing ATP levels and raising cyclic AMP levels within the sperm (16).

3. Acrosome Reaction

Due to the change in the membrane fluidity of the capacitated sperm head, the initiation of the acrosome reaction is triggered by the

fusion of the outer acrosomal membrane with the sperm head plasma membrane, releasing its contents. Subsequently, the acrosomal granule disintegrates, leading to the release of lysins (17).

These enzymes create a path by breaking down the zona pellucida (ZP), resulting in the fusion of the sperm head plasma and oocyte plasma membranes. The acrosome reaction exclusively occurs in the presence of Ca^{2+}, which may be induced artificially by adding Ca ionophore A23187. This chemical carries Ca^{2+} across cell membranes to the sperm cytoplasm or increases the external concentration of Ca^{2+} (18,19).
The process of acrosome exocytosis may entail the activation of multiple second messenger pathways, such as:

- Changes in intracellular Calcium
- Activation of cAMP and phosphokinase A pathways
- Phospholipase C zeta (PLCζ) generating Inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG)
- Phospholipase D generating phosphatidic acid
- Activation of phospholipase A2 generating arachidonic acid.

It is crucial to note that completing the acrosome reaction does not guarantee successful fertilization in vitro. For fertilization, the physiological processes that lead to the acrosome reaction must be parallel to those that activate the oocyte. These processes include changes in the ion permeability of the plasma membrane, alterations in the intracellular level of free Ca^{2+}, and alkalinization of the cytoplasm (20).

II. Oocyte Activation

1. Cytoplasmic Maturation

Throughout the stages of oogenesis, the oocyte has gathered stores of proteins and mRNAs, which enable it to stay quiescent, a state of developmental arrest marked by blocks at both the nuclear and cytoplasmic levels (21).
The germinal vesicle characterizes arrest during the first meiotic prophase. Following germinal vesicle breakdown, meiosis is again arrested, and the fertilizing spermatozoon removes this block. Two types of protein kinases maintain the second meiotic arrest in the cytoplasm: maturation-promoting factor (MPF) and cytostatic factor (CSF) (22).

Oocytes gain competence for successful fertilization and the ability to sustain early development through cytoplasmic maturation, which is analogous to sperm capacitation. Several developmental milestones must be met before an oocyte can be fertilized appropriately(19, 23):

- MPF is expressed at a high level. The core components of MPF are CSF and cyclin-dependent kinase1(Cdk1/Cdk2), CSF inhibits the anaphase-promoting complex/cyclosome (APC/C) via a signaling cascade involving early mitotic inhibitors, Emi2/Erp1.
- The oocyte contains elevated levels of various factors, such as c-mos, mitogen-activated protein kinase (MAPK), and active p34cdc2.
- Advancement to the MII phase of meiosis: The initial polar body must be expelled into the perivitelline space, which is located between the oolemma and the ZP.
- Transcription is almost completely halted by the time of germinal vesicle breakdown (GBVD). At this stage, gene expression transitions to the translation of stored mRNA.

In humans, the spermatozoon triggers an outward flow of electric current in the oocyte's plasma membrane by activating potassium channels controlled by Calcium. The activation competence of oocytes undergoes continuous changes in vitro and is not a stable or prolonged

characteristic of ovulated eggs. Therefore, precise timing is crucial when in vitro manipulations (24).

2. Cortical Reaction

Following sperm penetration, the calcium wave triggers the fusion of cortical granules with the oocyte plasma membrane, causing them to release their contents into the perivitelline space through exocytosis. The cortical reaction is the initial morphological sign of oocyte activation (25).

The cortical reaction elicits the zona reaction (zona hardening), changing the ZP characteristics. Concurrently, the oocyte plasma membrane becomes a mosaic of cortical granule and original plasma membranes. The fusion of the CG/oolemma leads to a transient increase in surface area, which allows for the required increase in metabolic turnover in the activated oocyte. The embryo remains within the protective zona coat until it hatches, just before implantation (26).

Intracellular Calcium Release

The calcium release pattern during fertilization exhibits species-specific variations. Mammals have two distinct calcium release mechanisms in oocytes (19, 27):

1. Inositol 1,4,5-trisphosphate- (IP3-) induced calcium release (IICR)
   IP3 is generated through the activity of phospholipase C on the lipid phosphatidylinositol bisphosphate in the plasma membrane. IICR is initiated by binding IP3 and its receptor (IP3R) on the endoplasmic reticulum.

2. Calcium-induced calcium release (CICR)
   CICR is activated by opening the ryanodine receptor on an intracellular store; however, it can also be induced via a mechanism involving the IP3 receptor. This release can also be triggered by Calcium, which cyclic ADP-ribose modulates. Consequently, cyclic ADP-ribose is produced by metabolizing nicotinamide adenine diphosphate (NAD+) by ADP ribosyl cyclase or NAD+ glycohydrolase.

The sensitivity to CICR and repetitive calcium spikes (oscillations) are significantly increased during fertilization. This indicates that both CICR and IICR are initiated during fertilization and that not only is cytoplasmic alkalinization required for fertilization, but also triggering the characteristic pattern of intracellular Ca^{2+} oscillations is mandatory for activating specific molecular pathways in the oocyte, resulting in meiosis resumption and completion (28).

All the molecular mechanisms associated with sperm and oocyte activation are essential for fertilization. Calcium release in such a specific pattern is the fundamental factor that induces these cascade events. Thus, in the case of OAD, the inclusion or induction of Ca release to augment AOA in vitro may be efficacious regardless of whether the failure of fertilization induction is caused by improper activation of sperm or oocyte (29).

The efficacy of AOA treatments is intricately linked to determining the causal factor behind FF. Several diagnostic tests have been devised to ascertain the etiology of OAD, such as heterologous and homologous tests, genetic tests, particle image velocimetry, and immunostaining (30).

Several strategies can be employed to raise calcium levels and stimulate fertilization. This can be achieved by directly introducing Calcium using substances like Ca^{+2} ionophore A23187 (calcimycin) or ionomycin. Alternatively, calcium oscillation release can be induced by natural stimulants such as phospholipase C-zeta (PLCζ) or by strontium chloride (SrCl2).

Artificial Oocyte Activation Approaches Based on Calcium Level

I. Direct Calcium Addition

The predominant approach for assessing AOA in humans involves the utilization of

ready-to-use Ca^{+2} ionophores, such as ionomycin and Ca^{+2} ionophore A23187. Specifically, following ICSI, all microinjected MII oocytes were incubated for 15 min in an ionophore and then washed; before transferring the injected oocytes to culture media droplets (31).

The application scope is to increase the fertilization potential by increasing the Ca^{+2} permeability of the cell membrane, thus enabling extracellular Ca^{+2} to flow into the oocytes; nevertheless, both Ca^{+2} ionophores cannot produce Ca^{+2} oscillations (32). Additionally, this method may prove ineffective if the injected spermatozoa lack sufficient levels of PLCζ to induce Ca^{+2} oscillations (33).

A retrospective cohort study by Miller et al. did not report a difference between ICSI and ICSI-Ca^{+2} in terms of the congenital disability rates (chromosomal aberration, structural malformations, and malformation types) and both singleton and twin pregnancies suggesting that in the event that ICSI fails or results in poor fertilization, oocyte activation with a Ca^{+2} ionophore could be an option (34).

Karabulut et al. demonstrated that the fertilization rates, embryos' quality, and pregnancy rates increased by adding Ca^{+2} ionophore, suggesting that patients with TFF and other patients with different indications may benefit from AOA (35).

Capalbo et al. found no evidence suggesting that AOA leads to an increase in errors in the process of chromosome segregation during meiosis. However, epigenetic defects could not be ruled out, implying that AOA should be used only in patients with specific indications and not in all cases (36).

On the one hand, combining ICSI with AOA using Ca^{2+} ionophores could benefit patients experiencing cleavage failure. This combination may assist the zygotes in progressing to more advanced developmental stages (37).

On the other hand, a randomized control trial revealed that the fertilization rate did not increase when AOA was applied using Ca^{+2} ionophore solution in patients with diminished ovarian reserve (38), In addition to its inability to induce calcium oscillations therefore became ineffective specially if spermatozoa lack PLCζ (33).

II. Induction of Calcium Oscillation Release

Strategies of AOA that aim to induce calcium oscillations are successful in overcoming FF caused by deficiencies in PLCζ-sperm (39).

The most common one is Strontium chloride, As it was observed that SrCl2 induces Ca^{+2} oscillations in mouse models, also it enhances fertilization rates, and improves embryo quality, when used in cases that experienced previous ICSI failure, leading to successful pregnancies (40- 41).

To understand the mechanism of how $SrCl_2$ induces oocyte activation; A recent study in mice reported that Sr^{2+} triggers the formation of transient receptor potential cation channels, subfamily V, vanilloid3 (member3) (TRPV3), mediating oocyte activation by promoting downstream oscillations in $[Ca^{2+}]i/[Sr^{2+}]$ of the oocytes, probably by sensitizing IP3Rs and thus facilitating Ca^{2+} oscillations, or substituting for Ca^{2+} in the potentiation of IP3Rs (42). The TRPV3 channels are strongly influenced by temperature (43) and can be regulated by various stimuli and ligands, including natural compounds, such as carvacrol, thymol, eugenol, and 2-amino ethoxy diphenyl borate (2-APB) (44).

The agonists 2-APB and carvacrol demonstrated their ability to stimulate TRPV3 channels and induce an influx of Ca^{2+}, activating mouse oocytes (42).

Moreover, compared to other agents, the efficacy of Sr^{2+} as an antioxidant method was highest in a mouse model with deficient sperm activation capacity (45) and, more recently, in a knockout mouse model for PLCζ. Given that Sr^{2+} elicits Ca^{2+} spikes similar to those caused by rodent sperm, Therefore, the TRPV3 channel antagonists, such as 2-APB and carvacrol, can potentially serve as an alternative AOA method in humans (44).

Fawzy et al. (2019) published an interesting randomised clinical trial that included 343 couples and sought to assess the impact of AOA in combination with either calcimycin or $SrCl_2$ on clinical pregnancy rates following ICSI. According to their findings, AOA in combination with either SrCl2 or calcimycin can increase the rates of clinical pregnancy, ongoing pregnancy, and live birth when compared to ICSI alone. Additionally, calcimycin AOA seems to be useful in overcoming sperm morphology defects, while $SrCl_2$ AOA seems to be more beneficial for ICSI cycles that have a history of poor fertilization (46).

Recombinant human PLCζ protein is a natural stimulant that triggers calcium oscillations. PLCζ is the primary spermatozoon protein activating the oocyte. Aberrant expression of PLCζ was noted in infertile men concerning ICSI failure, indicating that recombinant human PLCζ may be a potential treatment act as an activator or stimulant to improve fertilization after an ICSI procedure (47-48). The role of recombinant human PLCζ on AOA was demonstrated by Nomikos et al. who reported cytoplasmic Ca+2 oscillations during fertilization after recombinant human PLCζ protein administration, achieving mouse oocyte development to the blastocyst stage (49). Recently, it was discovered that injection of recombinant PLC ζ protein could effectively rescue mouse oocytes from activation failure in a mouse model of failed oocyte activation after ICSI (50). The same study revealed that Ca^{+2} signals can be restored using ionomycin. The restoration was more significant when the oocytes were exposed to strontium (Sr^{+2}) media or injected with PLCζ than those subjected to the Ca^{+2} ionophore.

On another hand, The correlation between sperm quality, specifically in relation to fertilization, and sperm DNA fragmentation has been extensively documented (51). The chromatin integrity is critical because the spermatozoon must deliver an intact genome to initiate the cell cycle and embryo division. Because the spermatozoon's fertilizing potential depends on PLC expression, it can be presumed that the percentage of DNA fragmentation affects PLCζ expression levels. Recent studies indicated a correlation between PLCζ and the status of sperm chromatin, suggesting that the percentage of DNA fragmentation could potentially impact AOD (52- 54).

However, despite the role and beneficial effect of human recombinant PLCζ, its routine use in IVF labs remains restricted due to its unavailability in the commercial market.

Artificial Oocyte Activation Approaches Not Based on Calcium Level

In contrast, deficiencies related to oocytes could be effectively addressed by utilizing alternative AOA promoters that trigger the deactivation of MPF and the resumption of meiosis. Such agents are comprised of cycloheximide, N, N, N', N'-tetrakis (2-pyridyl methyl) ethane-1,2-diamine (TPEN), roscovitine, and WEE2 (*oocyte-specific gene responsible in pronuclei formation during fertilization)* complementary RNA. Moreover, if OAD results from oocyte dysmaturity,

implementing a modified ovarian stimulation protocol and trigger could enhance fertilization (55).

A novel oocyte activation method involves using Zn^{2+} chelators to imitate the changes in intracellular Zn^{2+} levels during fertilization. This approach introduces a new way of designing methods for oocyte activation. The principle of using Zn^{2+} chelators for AOA is the fact that during fertilization, a rapid efflux of intracellular Zn^{2+} occurs, a phenomenon known as the "Zn^{2+} spark" (56), this phenomenon originates from the loss of cortical vesicles containing ~8 billion Zn^{2+} atoms through exocytosis (57) and is correlated to MPF inactivation and meiotic progression (58).

TPEN and other Zn^{2+} chelators can be used to simulate intracellular Zn^{2+} depletion (59- 60). Exploring Zn^{2+} chelator-mediated oocyte activation approaches introduces a novel method for activating oocytes that complements Ca^{2+} based activation methods synergistically to enhance oocyte activation.

Safety, Efficacy, and Ethical Issues

The cause of FF must be identified to improve the effectiveness and safety of AOA treatments. Although most data does not indicate any negative impact of AOA on the development of embryos before and after implantation, there is a lack of extensive research on this topic. Recent studies on mice propose that AOA may lead to epigenetic changes in the embryos and their offspring. Pending the availability of more comprehensive data and notwithstanding the promising outcomes achieved, applying AOA should be exercised cautiously in clinical settings and exclusively following adequate patient counseling. AOA should be regarded as a pioneering therapy rather than a well-established one (61).

Even though AOA is conducted as a routine procedure in numerous assisted reproduction centers, no relevant randomized-controlled trial exists to date. AOA with ionophores is currently used cautiously due to the possibility of epigenetic defects arising from its use. This is because while most Ca^{+2} agents can cause Ca^{+2} release, it is not at physiological levels (it could be sub- or supraphysiological). Nonetheless, this Ca^{+2} release triggers fundamental downstream events that may result in various gene expression patterns (62-63).

Furthermore, the observation that both gametes and preimplantation embryos exhibit a heightened metabolic rate, which could potentially govern the epigenetic process, may unavoidably result in epigenetic abnormalities (64).

Several assisted reproduction centers only use AOA in couples who have had multiple implantation failures after ICSI. There are still no follow-up studies on the health of newborns. Therefore further studies should be applied to investigate the effect of using AOA on the offspring to guarantee its safety (65).

Conclusion

OAD is a phenomenon that causes FF, which may be oocyte- or sperm-borne. Determining the cause of OAD is critical in selecting the right stimulant to overcome the detected defect and activate the other molecular events required for fertilization. AOA is not a routine procedure and should be introduced with caution for the ones who need and benefit from it.

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