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Fertilizasyonda Oosit ve Sperm Aktivasyon Faktörleri , Hücresel Mekanizmalar

Oocyte and Sperm Activation Factors and Cell Mechanisms in Fertilization

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Özet

Bu derleme ile amaç, sperm ve oosit kaynaklı aktivasyon faktörlerin fertilizasyondaki rolü ve fizyolojik yetersizliklerin nasıl tamir edileceği konusunda görüş oluşturmaktır. Öncelikle sperm kemotaksisi ve kapasitasyondaki mekanizmalar,kemotaktik ajanların rolü, sperm oolemma bağlanması mekanizmalarını açıklıyoruz. Daha sonra ise sperm kaynaklı faktörlerin membran füzyonunda nasıl görev yaptıkları ve hangi mekanizmaları uyardığı anlatılacaktır. Oosit mayozunun ileri aşamaya geçişinde etki yapan faktörler ve sitoplazmik mekanizmalar üzerinde durulacaktır. Kortikal granül salgılanması 2. PB atılması ve pronukleus gelişimi ile ilişkili mekanizmalar, bu konuda yapay oosit aktivasyonlarının önemi üzerinde çalışılan konulardır. Sperm proteinleri, sperm spesifik bir fosfolipaz olan fosfolipaz C ζ'yi (PLCζ) ve post-akrozomal WW bağlayıcı alan proteini (PAWP/WBP2NL) ile birlikte oosit aktivasyonu, depolanmış kalsiyum'un salınımındaki mekanizmalar, CatSper membran katyon kanal proteinleri,P3R, Protein kinaz C ve oosit faktörleri ile etkileşim ve bu mekanizmalardaki aksamalar, çözüm önerileri, muhtemel stratejiler değerlendirilecektir.

Anahtar kelimeler: Oosit aktivasyonu, sperm proteinleri, fertilizasyon

Abstract

The purpose of this review is to develop a perspective on the role of sperm- and oocyte-derived activation factors in fertilization and to explore how these factors can be used to correct physiological deficiencies. First, we explain the mechanisms of sperm chemotaxis and capacitation, the role of chemotactic agents, and sperm-oolemma binding. Next, we will explain how sperm-derived factors function in membrane fusion and the mechanisms they stimulate. We will focus on the factors and cytoplasmic mechanisms that influence the transition to advanced oocyte meiosis. Cortical granule secretion, 2nd PB shedding, and mechanisms associated with pronucleus development are among the topics studied in this regard, highlighting the importance of artificial oocyte activation. The sperm proteins, sperm-specific phospholipase C ζ (PLCζ), and the post-acrosomal WW-binding domain protein (PAWP/WBP2NL), will be evaluated for oocyte activation, mechanisms involved in the release of stored calcium, interactions with CatSper membrane cation channel proteins, P3R, protein kinase C, and oocyte factors, and disruptions in these mechanisms, proposed solutions, and potential strategies.

Keywords: Oocyte Activation, Sperm Proteins, Fertilization

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1. Introduction

Fertilization biology provides essential information for studying cell proliferation and physiology (1,2,3). Cell cycle research and cell-cell interactions are crucial steps in this process and help explain infertility (4,5). The oocyte's dwell time in Metaphase 2, increases in intracellular Ca++, and factors influencing related mechanisms are vital for fertilization success (6,7). Chemotaxis, a physiologically necessary action, is observed in many cells, including spermatozoa, aiding them in reaching the oocyte (8). Chemotactic agents differ among species. In human oocytes, zona proteins, hyaluronic acid, and progesterone serve as primary chemoattractants (9,10). Human sperm gain fertilization capacity after capacitation, which triggers events such as membrane protein regulation, activation of intracellular cAMP-dependent processes, and increased membrane permeability (11). After capacitation, sperm penetrate the cumulus cells with hyperactivated motility and bind to the zona. Following attachment to the zona pellucida, they fuse with the cell membrane in the perivitelline space, aided by the calcium-associated acrosome reaction and the release of proteolytic enzymes (9,10,11). Sperm Ca++ channels, CatSper, and zona proteins participate in processes like the acrosome reaction and hyperactivated motility (12,13). Multiple factors are believed to influence sperm capacitation and acrosomal reactions, although the exact mechanisms remain unclear (14). It is widely believed that proteins such as phospholipase C zeta or WBP2 N-terminal-like (WBP2NL or PAWP), secreted by sperm entering the oolemma, may activate the oocyte (15). Oocyte activation induces cytoplasmic events, including the formation of female and male pronuclei, cortical granule exocytosis, and the start of cleavage (17). This review discusses the mechanisms outlined above and the role of artificial oocyte activators in fertilization biology and IVF clinics.

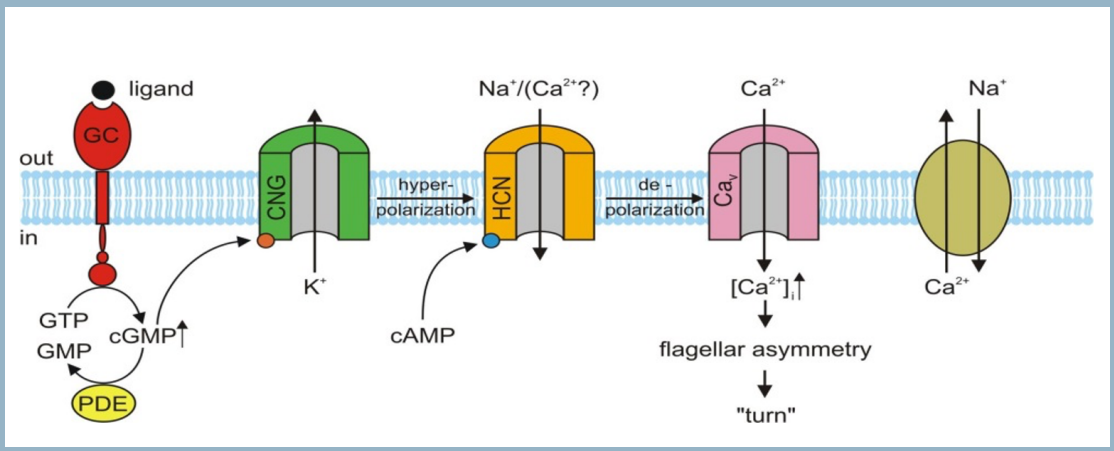

Figure 1. A model of the signal transduction pathway during sperm chemotaxis in the sea urchin Arbacia punctulata. (Kaupp, U.B., 2006, 41)

Chemotaxis

Chemotaxis is the movement of sperm toward a chemical gradient. Cumulus granulosa cells surrounding the oocyte undergo luteinization and secrete progesterone, and picomolar P4 secretion can induce human sperm migration (18). It has also been shown that chemotactic agents activate signaling mechanisms through various pathways. For example, several proposed mechanisms include P4 signaling, which initially activates the tmAC-cAMP-PKA pathway, followed by protein tyrosine phosphorylation and Ca++ mobilization, as well as membrane hyperpolarization after activation of the sGC-cGMP-PKG cascade (19,20). Ultimately, sperm move toward the chemotactic agent. The signal transduction model for sea urchin sperm chemotaxis involves the binding of the chemotaxant to a membrane-bound guanylyl cyclase, which activates cGMP synthesis from GTP, opening cyclic nucleotide-gated potassium channels and causing membrane hyperpolarization (21). After hyperpolarization, hyperpolarization-activated cyclic nucleotide-gated (HCN) channels allow Na+ influx, leading to depolarization and a rapid influx of Ca+ through voltage-activated Ca+ channels (Cav). The Ca+ ions interact with the sperm flagellum's axoneme via unknown mechanisms, increasing the asymmetry of the flagellum's beat and causing a rotation or bend in the swimming path. Ca2+ is then removed from the flagellum by a Na+/Ca+ exchange mechanism.

Sperm Capacitation

The capacitation process, which is an essential step in gaining the fertilization ability of sperm, causes both the acrosomal reaction and hyperactivation motility (22). During capacitation, an increase in intracellular bicarbonate (HCO3-) is observed, following the acrosome reaction triggered by adenyl cyclase, cAMP, and Protein Kinase A activation, along with modifications in cholesterol within the membranes and its mode (23). Throughout this process, the sperm membrane potential is mainly hyperpolarized due to CatSper activation, leading to the release of potassium ions (K+) (23). A sperm undergoing capacitation exhibits hyperactivation motility and undergoes acrosomal exocytosis by passing enzymes through the zona pellucida and chemically dissolving the zona (24). An intracellular rise in Ca2+ in sperm is crucial for initiating hyperactivation motility and the acrosome reaction, which are essential physiological events for fertilization (24,25). Sperm stimulation and activation of G-protein-dependent receptors on the sperm plasma membrane occur. Protein kinases facilitate the phosphorylation of proteins, which subsequently trigger the acrosomal reaction (24,25). Capacitation induces changes in intracellular metabolism, including increased tyrosine phosphorylation, pH, cAMP, and reactive oxygen species (ROS), making spermatozoa capable of fertilizing (26).

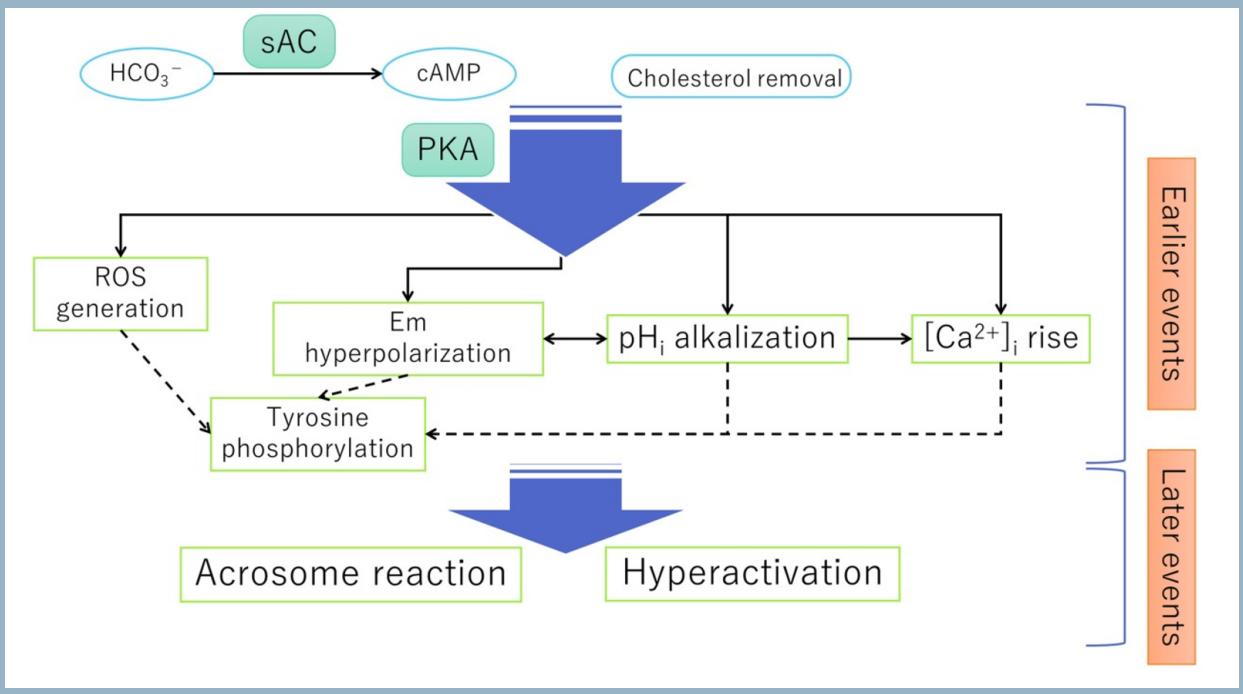

Figure 2. Summary of molecular mechanisms in sperm capacitation. ROS generation causes membrane hyperpolarization, higher intracellular pH, increased Ca++, and hyperactivation and acrosome reaction due to tyrosine phosphorylation of flagellar proteins. These events are regulated downstream of the cAMP/PKA pathway, which is triggered by HCO3– exposure and the removal of cholesterol from the plasma membrane by soluble adenylyl cyclase (sAC). cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; ROS, reactive oxygen species; Takei, G. L. (2023, 42).

Sperm-Oocyte Binding And Acrosome Reaction

In capacitation, cellular preparation ensures that sperm are bound to the zona and the acrosomal reaction occurs. The disruptions that happen in connection with the zona are the cause of infertility in men. The sperm connected to the zona have higher HSPA2 and SPACA3 expression levels (27). This finding suggests that the Zona Pellucida may be able to select better sperm. The same study explains that these sperm also exhibit higher motility and better morphology, as well as higher protamination and DNA integrity (27). This situation is similar to our results (28,29). In our study, we found that chromatin condensation and DNA integrity are higher in cases where sperm morphology and motility are better (28,29). There are studies on modifications of sperm membranes during capacitation and the acrosome reaction. In this context, a study in rats emphasizes the importance of molecular modifications of MC31/CE9, a sperm surface molecule, during sperm capacitation and the acrosome reaction (30). At the end of these processes, various proteins related to sperm and eggs are involved in the fusion process gamete.

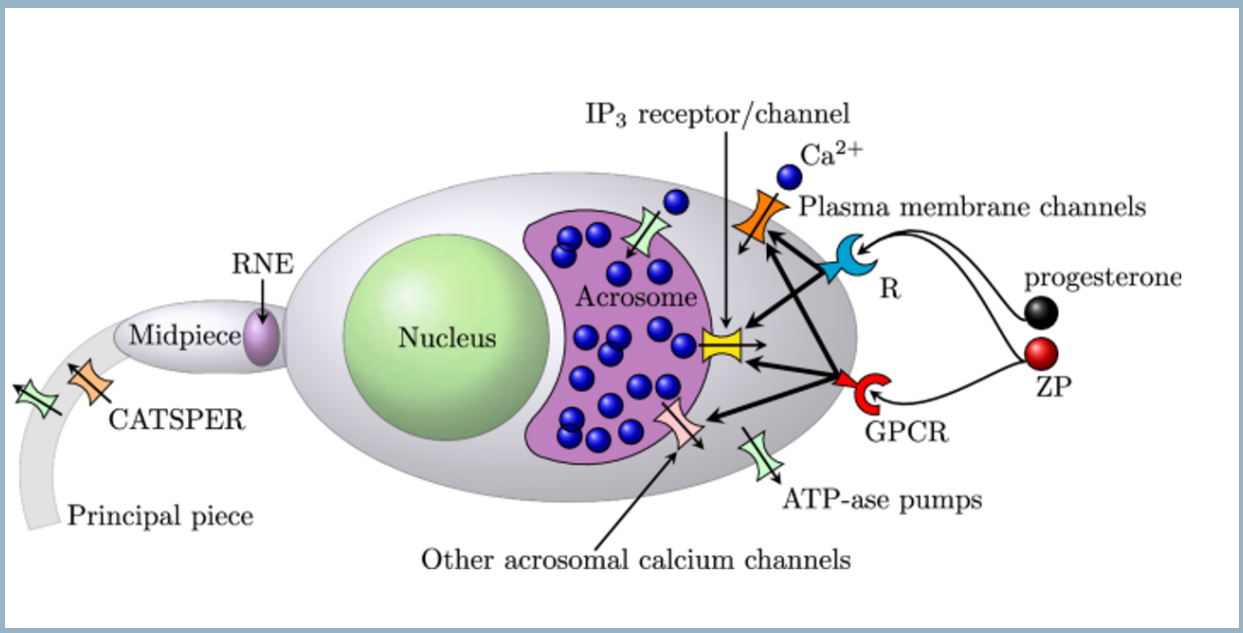

Figure 3. A drawing of the acrosomal response to stimulation from the sperm head and signals such as zona pellucida glycoprotein (ZP) or progesterone (P4). Small arrows indicate the direction of calcium influx through the channels, while stimulation of the channels is indicated by thicker black arrows from receptors (R or GPCR). GPCR represents a G protein-specific receptor, while R represents a pertussis toxin-insensitive receptor. ATPase pumps are shown in green, receptors/channels in yellow, outer membrane channels (plasma membrane, CATSPER) in orange, and other acrosomal calcium channels in pink. Arrows passing through the pumps indicate calcium transport. (Simons, J., 2018, 43)

Ca++ ions and cytoplasm alkalinization play a significant role in sperm capacitation (32). Calcium levels increase in two ways: first, from calcium stores during the acrosomal reaction, and second, through Ca++ channels (CATSPER) (33). Ca++ channels activate when the rise in intracellular calcium in sperm is insufficient (32). Catsper and HV1 channels are specific to human spermatozoa (34,35). Studies have shown that Catsper channel activity is influenced by albumin, basic pH, zona proteins, and hyaluronic acid (36).

Sperm Activation Factors

Spermatozoa binding to the egg causes an increase in intracellular calcium levels by facilitating the action of acrosomal enzymes. Calcium increases Sperm Oocyte Activation Factor, which activates Phospholipase C zeta and produces inositol triphosphate and diacylglycerol in the cytoplasm, both of which activate inositol triphosphate receptors and protein kinase C, leading to increased sperm Ca++ (32). However, recent studies suggest that WBP2 N-Terminal-like (WBP2NL or PAWP) may also be a candidate protein outside Plczeta (17). Currently, both PLCζ and PAWP are the leading candidates for ovocyte activation during fertilization. Although IP3 mediates the PLCζ mechanism, how PAWP activates the ovocyte remains unclear. These findings are important for understanding and treating fertilization failure due to oocyte activation, especially when caused by PLCζ deficiency in sperm. However, there is no routine laboratory test to detect PLC presence. It has long been known that paternal age can reduce sperm activity (37). Japanese researchers have identified a new sperm factor, demonstrating that sperm failed to fertilize the oocyte when citrate synthetase activity was insufficient (37).

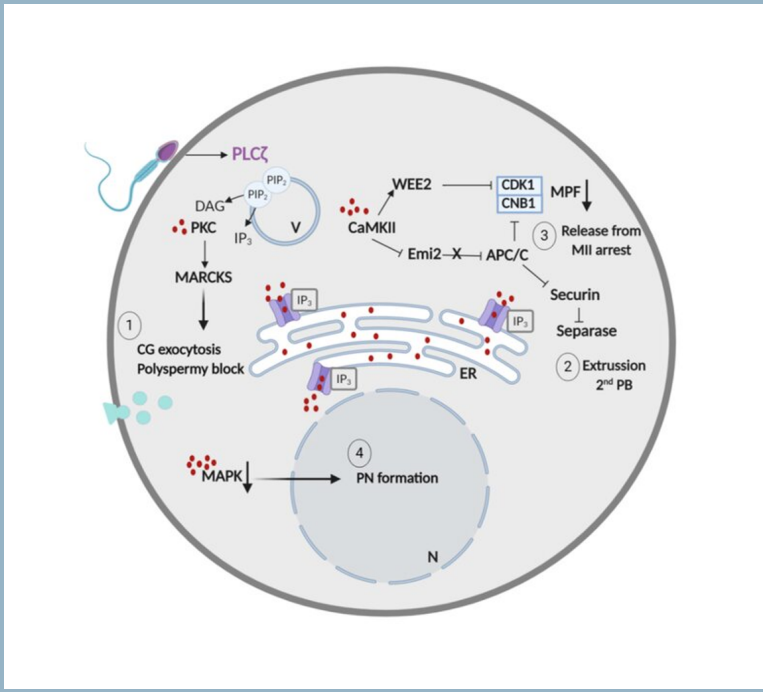

Fig. 4. Oocyte activation pathway triggered by sperm factor PLC□. Sperm PLC□ released into the oocyte induces IP3 production, which then binds to its receptor (IP3R) and causes Ca2□ release from the endoplasmic reticulum. Ca2□ oscillations activate various oocyte kinases over time, enabling cortical granule (CG) exocytosis through protein kinase C (PKC) activation, meiotic resumption via second polar body (PB) shedding and Ca²□/calmodulin-dependent protein kinase II (CaMKII) activation, and pronucleus formation (PN) through Mos/mitogen-activated protein kinase (MAPK) inactivation. PLC□: phospholipase C zeta; DAG: diacylglycerol; PIP2: phosphatidylinositol 4,5-bisphosphate; IP3: inositol 1,4,5-triphosphate; PKC: protein kinase C; MARCKS: myristoylated alanine-rich C-kinase substrate; WEE2: Wee1-like protein kinase 2; CDK1: cyclin-dependent kinase 1; CNB1: cyclin B; MPF: maturation-promoting factor; Emi2: early mitotic inhibitor 2; APC/C: anaphase-promoting complex/cyclosome; V: vesicle; N: nucleus; ER: endoplasmic reticulum; MII: metaphase II. (Barberan A.C.,2020,44)

Assısted Oocyte Actıvatıon And Oocyte Actıvatıon Faılure: Solutıons

ICSI overcomes numerous obstacles, such as capacitation, hyperactivation, and the acrosomal reaction. Mechanical oocyte activation is achieved through ICSI.

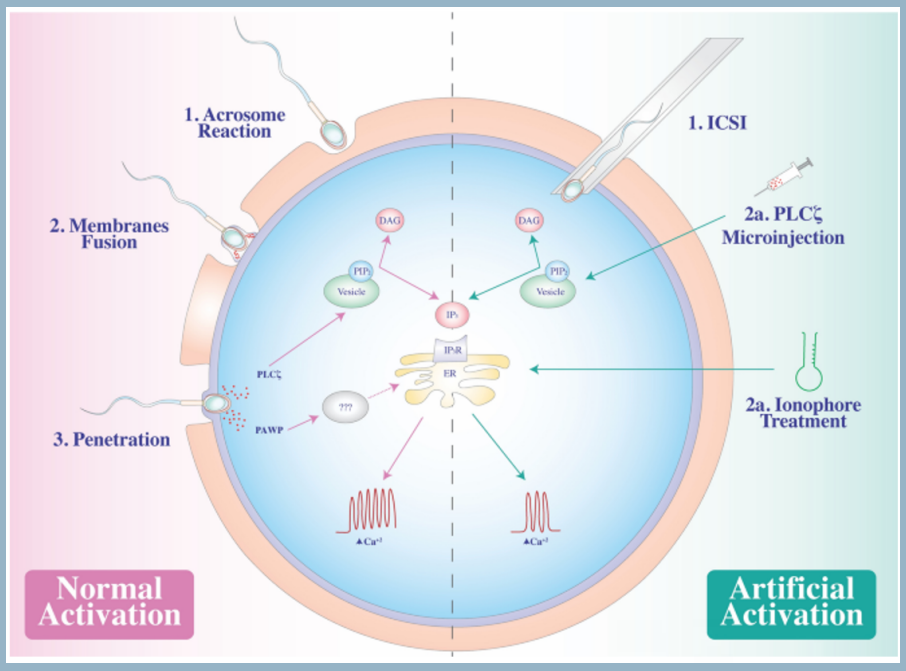

Figure 5. The role of sperm factors in three different types of oocyte activation. During normal activation, the acrosome reaction (1) begins at the equatorial segment and continues to the postacrosomal sheath-perinuclear theca (PAS-PT), exposing the PT (2) for the sperm membrane to fuse with the oocyte (3), leading to penetration. SOAFs are released into the ooplasm and trigger Ca2+ release via IP3. In artificial oocyte activation, various strategies following ICSI can induce Ca2+ oscillations, such as PLC microinjections (2a) or ionophore treatments (2b) (Zafar, 2021, 17).

Numerous studies have demonstrated that phospholipase C zeta, as a sperm-oocyte activating factor, is involved in oocyte activation—including MII oocyte activation via inositol-1,4,5 triphosphate (IP3), meiosis progression, and pronucleus formation (17,32,37). Research indicates that PLCζ is the most critical sperm-oocyte activating factor. It induces Ca2+ oscillations through PIP2 hydrolysis to produce IP3 and diacylglycerol. It binds to IP3 receptors on the ER surface, triggering wave-like Ca2+ oscillations. In 2007, the PAWP protein was also shown to have oocyte-activating properties (36). Both factors promote Ca2+ oscillations, cortical granule release, and pronucleus formation during fertilization (32,36,38,39). Despite these findings, data on the PAWP protein suggest that further studies are necessary.

Fertilization failure (TFF), characterized by the inability of all oocytes to form pronuclei, indicates a defect in oocyte activation (17). Artificial activation agents have been developed to address this issue, with Ca2+ ionophore compounds being the most widely used (35). Animal studies have demonstrated that injections of ionophore A23187 and its analogue, ionomycin, support embryo development up to the blastocyst stage and enable parthenogenetic embryo formation (40). In 2017, Murugesu et al. reported that using Ca2+ ionophore during ICSI significantly improved oocyte activation and pregnancy rates (40). Alternatively, treatments involving pharmacological, chemical, or external agents such as PLCζ microinjection are available (39). However, their clinical application remains limited. Mechanical, chemical, and electrical activation methods target the oocyte's endoplasmic reticulum and Ca++ ion release (35,36,37).

Conclusions

Many aspects of sperm chemotaxis, capacitation, and the acrosomal reaction are still not fully understood. Controlling the influx of Ca++ ions across membranes and the increase in intercellular Ca++ levels involves numerous unknowns. It is evident that further research into the hyperactivation of voltage channels (HV1), the alkalinization of sperm cytoplasm, and the role of CatSper cation channels is necessary. The effects of new sperm-oocyte activation factors also warrant investigation. Sperm factors such as PLCζ and PAWP, along with oocyte proteins like CAMK-II and other kinases, are crucial for advancing infertility treatments. Gaining a clearer understanding of sperm ion channels and transporters will help elucidate sperm motility, physiological function, and Ca2+ homeostasis regulation. Although it was previously thought that only one sperm factor was responsible for oocyte activation, recent findings suggest citrate synthase can also serve as a sperm factor. Nonetheless, further research is essential to clarify the intra-oocyte mechanisms.

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