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Oocyte Metaphase Arrest and Release: Triggers and Pathways

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Abstract

Release of a mature egg is an important pre-requisite of mammalian reproduction. Starting from the time when oogenesis begins during fetal development, to the time of puberty and until fertilization, the oocyte encounters several stop-and-go periods. Altered pathways may affect fertilization outcome. Understanding of the stop-and-go periods is based on exploring the underlying mechanisms. This review aimed at addressing the triggers and signalling pathways leading to oocyte meiotic II resumption. A detailed literature search was done for studies on various databases such as Google, PubMed/MEDLINE, Cochrane reviews etc.with related keywords such as oocyte meiotic release, oocyte metaphase arrest, oocyte activation, sperm oocyte activating factors etc. Selected studies were reviewed by two observers. All obtained information was analysed and was shaped into manuscript appropriately. It was explained that till ovulation begins oocytes are held in meiotic arrest in diplotene stage of prophase I. Just before ovulation luteinizing hormone (LH) surge directs resumption of meiosis I. The arrested primary oocytes complete meiosis I and progresses through second meiotic division and gets arrested again at metaphase II (MII) until fertilization. Results concluded that the MII arrest persists till sperm enters the oocyte and releases its activating factors into the ooplasm. These activating factors are the triggers unlocking the subsequent pathways releasing oocyte from meiotic arrest.

Keywords: Sperm, Oocyte activation, Calcium oscillations, Metaphase arrest

Introduction

Oocyte activation is a vital event during fertilization as failure or incomplete oocyte activation ends up in infertility (1,2). Oocytes are arrested at diplotene stage of prophase I until ovulation begins. LH surge triggers oocyte meiotic resumption followed by arrest at MII. Oocytes passed through meiotic I arrest and prevent their parthenogenetic activation by arresting again at MII. The MII arrest endures till sperm oocyte fusion occurs. Fertilization is a complex phenomenon that involves cellular changes both in oocyte and sperm components. It encompasses step-wise interaction of sperm with oocyte and its outer coverings: cumulus cells, zona pellucida and oocyte plasma membrane. A mature sperm is well equipped to pass through these barriers. As soon as sperm fuses with oocyte membrane there is release of sperm oocyte activating factors (SOAFs) into the ooplasm. The released contents trigger the signal transduction events leading to MII completion and formation of the pronuclei. Oocyte intracytoplasmic calcium (iCa2+) rise plays a very crucial role in oocyte meiotic resumption. Ca2+ rise occurs within one to three minutes of sperm-oocyte fusion. The iCa2+ rise further upregulates the downstream signalling pathways stimulating oocyte to resume and complete MII.

Methods

A comprehensive literature search was done for studies on various databases such as Google, PubMed/MEDLINE, Cochrane reviews) with related keywords such as oocyte meiotic release, oocyte metaphase arrest, oocyte activation, sperm oocyte activating factors etc. Studies with full text available online were selected. The inclusion criterion was accessing the full text of the articles, and the exclusion criterion was conference papers and articles, articles full-text versions of which were unavailable. Research on oocyte meiotic arrest and release published in national and international journals was the main focus of the study. Articles related to the signalling pathways leading to oocyte activation were included in the research as well. Finally, the findings were classified into major areas including the triggers, events of oocyte meiosis completion and role of assisted oocyte activation. Selected studies from 1980-2020 were reviewed by two observers.

Results

Our literature search selected around 50 studies based on which we conceptualized our manuscript. The review included most complex areas of oocyte arrest and release. The major outcomes of the review are depicted in table 1.

The Triggers

Data from various studies have shown that sperm releases the triggering factors that elicit subsequent Ca2+ oscillations in the ooplasm. These triggers are known as SOAFs. SOAFs mediated rise in iCa2+ leading to oocyte meiotic resumption is well established. Many SOAFs have been discovered such as oscillin, PLC-zeta (ζ), PAWP, TR-kit but till now none of them found to have a convincing role.

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PLC-ζ: Till now PLC-ζ has been most widely studied and accepted as SOAF. It belongs to phospholipase C family. It was first identified to trigger Ca2+ oscillations in mouse eggs. The smallest PLC isoform, consists of C2 domain, four tandem EF-hand domains, and X and Y catalytic domains. Each of these domains known to play a crucial role in PLC-ζ function. PLC-ζ is located in the acrosomal, equatorial, post-acrosomal region of sperm head. But there have been recent studies questioning its role as the only SOAF suggesting possibility of other novel molecules contributing for rise in iCa2+ (3,4).

Oscillin: Oscillin was identified in hamster sperm as a factor eliciting Ca2+oscillations. Oscillin like protein has been localized to equatorial region of human sperm (5). One study has shown that sperm cytosol contains 33 kDa protein that was reactive with anti-oscillin hamster antibody (6). Oscillin also showed weak cytoplasmic staining in globozoospermic sperm (7). Membrane damage also correlated with oscillin localization (8). It was also found that it showed high level of homology with the bacterial enzyme glucosamine-6-phosphate isomerase suggesting that it might play more fundamental roles such as in carbohydrate metabolism. Though oscillin is a housekeeping gene conserved throughout evolution but it is not the main sperm-specific factor responsible for Ca2+oscillations. Moreover, there were not many studies available on the role of oscillin.

PAWP (Post-acrosomal WW-binding domain protein): PAWP is localized in the post acrosomal region of sperm head. The structure consists of N and C-terminals; N terminal has sequence homology to WW-domain binding protein 2 and a proline-rich C-terminal with a PPXY consensus binding site and an unknown repeating motif. It is thought to bind to oocyte-borne YAP proteins, and subsequently interacts with the SH3 domain of phospholipase C, leading to activation of the phosphoinositide signalling pathway. PAWP was unable to rescue failed oocyte activation in infertile male with homozygous mutation of PLC-ζ (9).

Truncated c-kit tyrosine kinase (Tr-kit): This protein consists of 202 amino acids and contains phosphotransferase catalytic domain of the c-kit cytoplasmic portion (10). Tr-kit lacks the phosphotyrosine docking site for interacting with the 85 kDa subunit of inositol 3-phosphate kinase (PI3K) (11). But it contains the carboxyterminal region for interaction with phospholipase C γ (PLCγ) (12). It was shown that tr-kit is localized in the residual sperm cytoplasm with the highest concentration in the midpiece. Microinjection of this protein into oocytes causes complete parthenogenetic egg activation and activation is inhibited by intracellular Ca+2-chelating agents or by specific inhibitors of PLC activity (13).

Citrate synthase: Citrate synthase (CS) is a mitochondrial enzyme present almost in every cell of the body and help in the condensation of citrate from oxaloacetate and acetyl Co-A. It plays an important role in carbohydrate metabolism as rate-limiting step during kerbs cycle (14). CS also inversely cleaves citrate to produce acetyl CoA and oxaloacetate which were proposed to cause egg activation in newt eggs. In newts, CS released from sperm can induce Ca2+ oscillations. It was shown that Ca2+ increase from mitochondria and ER is caused by oxaloacetate and acetyl-CoA respectively (15). It was also shown that CS exhibits higher activities in mature sperm and seminal quality correlates with CS activity (16). Recently the role of extramitochondrial CS located in sperm head has been identified in eliciting oocyte activation in mice (17).

The Events

Oocyte activation involves a series of morphological and biochemical events beginning with repeated Ca+2 oscillations (electrical events) leading to meiotic resumption, cortical granules exocytosis (structural events) and block to polyspermy. These changes constitute early events followed by the late events such as de-condensation of sperm nucleus, recruitment of maternal RNAs, second polar body extrusion, pronuclei formation, DNA synthesis and process of cleavage (18,19). Therefore, rising Ca+2 levels leading to meiotic resumption is the first sign of oocyte activation. In this review signalling pathways of oocyte MII arrest and release are discussed in detail.

Oocyte MII arrest

Oocyte MII arrest is maintained by the 'Cytostatic Factor' (CSF) that blocks the exit of arrested oocyte until the sperm breaks it by generating oocyte cytoplasmic Ca2+ oscillations. CSF is not a single molecule but it comprises of an activity that inhibits cell division and keeps the oocyte arrested in MII (20). CSF maintains arrest by preventing loss in Maturation-Promoting factor (MPF) and Emi2 (Early mitotic inhibitor 2) mediated inhibition of Anaphase-Promoting Complex/Cyclostomes (APC/C) (21). MPF is a complex of two subunits: cyclin dependent kinase 1(CDK1)/cyclinB. CDK1 is the catalytic subunit and cyclinB is the regulatory subunit. CDK1 has no catalytic function without its regulatory partner. At the time of meiotic arrest, there is high activity of MPF. Egg specific protein Emi2 is a substrate for polo-like kinases (plk) and usually undergoes Ca2+ dependent degradation. APC/C is a ligase that helps in polyubiquitination of key cell cycle proteins such as cyclin B, targeting them for immediate proteolysis and degradation. During oocyte MII arrest, Emi2 inhibits the APC/C complex. Another kinase WEE1B remains deactivated thereby stabilizing CDK1. This leads to maintenance of MPF activity and meiotic arrest (22).

There is also role of spindle assembly checkpoint (SAC) proteins suggested in maintaining CSF activity. The SAC proteins inhibit APC/C (23). The suggested proteins are Bub1, Mad1, Mad2. These SAC proteins are the downstream effectors of c-Mos/MEK/MAPK pathway. c-Mos is a kinase belong to proto-oncogene family regulating cell growth and development (24). c-Mos activates MAPK kinase MEK1 that further activates MAPK which subsequently activates the SAC proteins (25). The c-Mos/MEK/MAPK signalling pathway is an ideal CSF candidate as it has shown to stabilize and activate MPF (26). Thus, oocyte meiotic arrest is maintained by the CSF activity which comprises of MPF stabilization and APC/C inhibition through Emi2, WEE1B and SAC proteins (Figure 1 A).

Oocyte meiotic II release

During fertilization sperm releases SOAFs such as PLC-ζ in the oocyte that hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). Subsequently, IP_3 binds to specific receptors InsP3Rs present on smooth endoplasmic reticulum (SER). InsP3Rs is a protein complex of four subunits. Each subunit can bind to one IP_3 molecule leading to the subsequent release of Ca^{2+} from SER store.

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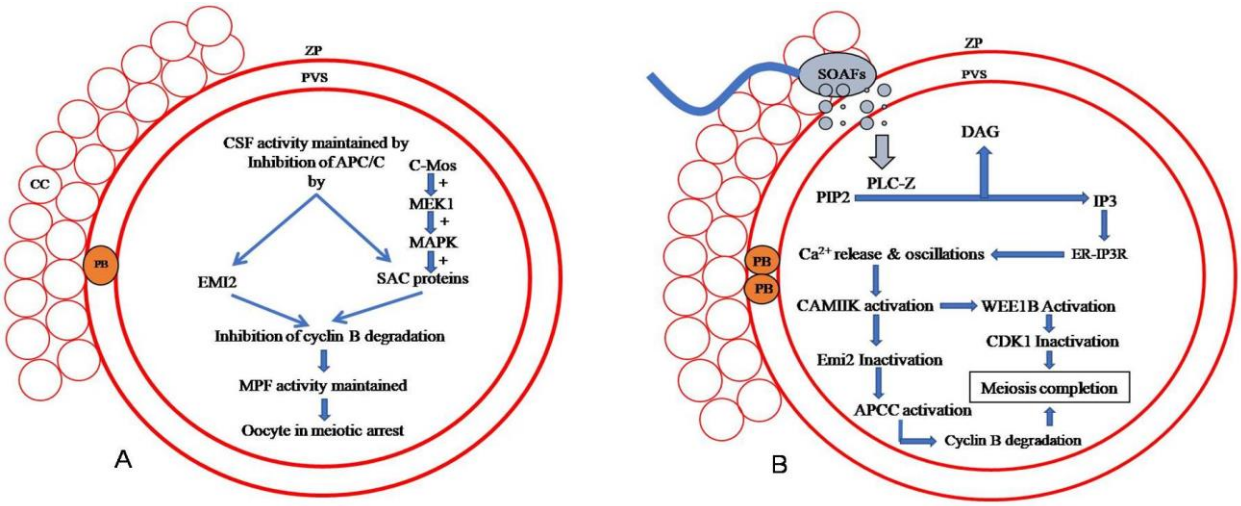

Figure 1. Mechanism of oocyte meiotic arrest (A) and release (B) .ZP: zona pellucid; PVS: perivitelline space; CC: cumulus cells; PB: polar body; CSF:Cytostatic Factor; MPF: Maturation-Promoting factor; Emi2:Early mitotic inhibitor 2; CDK1:cyclin dependent kinase 1; APC/C: Anaphase-Promoting Complex/Cyclostomes; SAC: spindle assembly checkpoint

The sensitivity of InsP3Rs is biphasic and is gated by Ca^{2+}. Thus, regulation of the receptor is complex: it is opened by IP_3 but is also desensitized by it, while low and high Ca^{2+} concentrations make it relatively insensitive to otherwise activating IP_3 levels. Ca^{+2} is loaded in the lumen of SER by Ca^{2+} ATPases (SERCA pumps) and stored while attached to Ca^{2+}-binding proteins. During signaling this stored Ca^{2+} is released into the cytosol by Ca^{2+}- release channels. This spike and fall of Ca^{+2} levels in the ooplasm elicit Ca^{+2} oscillations (27).

Apart from ER, the other organelle vital for oocyte maturation is mitochondria that is not only an essential energy source but also take part in maintaining Ca^{+2} oscillations (28,29). Ca^{+2} enters the cytosol from ER, which in turn opens one of the ER channels, sarcoplasmic reticulum/ER Ca ATPase (SERCAs), to transport Ca^{+2} back to the ER. The cytosolic Ca^{+2} is also taken up by the neighbouring mitochondria. The mitochondrial Ca^{+2} activates mitochondrial metabolism and production of ATP (30). This ATP is essential for the Ca^{+2} pumps/SERCAs maintaining the Ca^{+2} levels in the oocyte cytoplasm and in the ER (31,32).

Ca^{2+} oscillations switch on the CaMKII (calcium/calmodulin-dependent protein kinase II) (33). Activated CaMKII in turn phosphorylates the oocyte-specific protein Emi2 leading to its inactivation. The inactivated Emi2 no longer will be able to inhibit APC/C. APC which was inactive during arrest is now liberated from the inhibition by CSF. APC activation causes a decline in MPF activity by degradation of cyclin B and therefore the concomitant release from the MII arrest. Ca^{2+} oscillations also prevent MPF levels to return to active levels thereby helping in successful meiotic resumption. As such inhibition of CDK1 may also lead to release of MII arrest but sperm mediated Ca^{2+} signalling mediates loss of cyclin B via upregulation of APC/C rather than CDK1 inhibition (34). The other way by which CAMKII regulate MPF activity is through phosphorylation and activation of the protein kinase WEE1B (22). Phosphorylation of WEE1B deactivates CDK1 and leads to resumption of meiosis (Figure 1 B).

Releasing oocyte MII arrest by assisted oocyte activation (AOA)

Assisted oocyte activation (AOA) is a suitable technique for fertilization rates below 30% following conventional ICSI (35-37). The methods used for artificially activating oocytes are mechanical, chemical or electrical (38,39). Mechanical activation involves vigorous cytoplasmic aspiration before injecting the sperm (40). Direct current voltage generates electric field by moving charge proteins in lipid bilayer of membrane. This electrical activation involves pores generation in the plasma membrane thereby involving Ca^{+2} influx from the surrounding media. Ca^{+2} concentration increases immediately after electrical stimulus and subsequently decreases and return to original concentration without further oscillations. The success rates of electrical activation are lower than chemical activation methods (40). Chemical activation is the most common method used where oocytes are exposed to activating agents that leads to rise in Ca^{+2} levels. These agents can cause single or multiple oscillations. Common activating agents causing single transient are calcimycin and ionomycin (41). These increase the membrane permeability of plasma membrane to Ca^{+2} causing extracellular Ca^{+2} to flow into the oocytes. Use of calcium ionophores have shown increased pregnancy rates in recent studies but long-term effects on babies born out of ICSI-AOA have not been shown (42,43).

Chemical activators that cause multiple transients are strontium chloride (SrCl2), phorbol esters, anhydrous alcohol etc (44,45). SrCl2 is the most commonly used agent in mice but its use in human is still questionable. Moreover, mechanism by which SrCl2 releases Ca^{+2} is also not searched much. Safety of artificial activators has been an area of much discussion (39). Overall registration of number of births after AOA is still very low. It has been reported that use of calcium ionophores has been found safer and has not caused any bad effect on child health (37). There is only one study that reported a baby born after calcimycin AOA with anal atresia (46). A recent study investigated the subsequent health of children born after AOA (47). There was no significant

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difference found between AOA-ICSI and conventional ICSI in terms of birth weight, growth rate, preterm births etc. There were no chromosomal anomalies. Data related to SrCl2 studies are less consistent. Mice studies showed reduced birth weight of pups (48). In one of the studies, there was no oscillations observed following SrCl2 mediated AOA. Few studies in humans have shown no impact on physical and mental development of children born from AOA from birth till 4 years (44,49).

Although data reported till now are encouraging, AOA yet to be considered completely safe and effective as the number of studies and sample sizes were too less. Mechanism and clinical effects of AOA agents needs to be validated in larger studies. Data reported on fertilization and pregnancy rates are also variable. Moreover, sufficient data on follow up of children born following AOA remain deficient. AOA protocols in different reports differ in ionophore concentration, duration and number of exposures. The question of mechanism of action of AOA agents and downstream signalling pathways remains unanswered. The effects of calcium ionophores on gene expression remain to be studied. It has been found that risks of imprinting errors are more with conventional ICSI than normal pregnancy (50). Similar effects in ICSI-AOA are yet to be explored. Pattern of Ca^{+2} oscillations generated in IVF and ICSI is different from those produced by AOA agents; therefore, their potential side effects are of great concern (51). Further research is needed to assess these effects with use of AOA agents. Other definite options will be use of purest form of recombinant PLC-ζ or other sperm factors with proven oocyte activation potential.

Until use of them is passed through clinical trials and adequate scientific evidence is obtained regarding their safety, till then ICSI with AOA remains a debatable issue.

Table 1. Major factors regulating Oocyte meiotic events

Triggers	Events	Assisted Oocyte Activation
PLC-ζ (3,4) Oscilin (5-7) PAWP (Post-acrosomal WW-binding domain protein) (9) Truncated c-kit tyrosine kinase (Tr-kit) (10-13) Citrate synthase (14-17)	**Oocyte MII arrest** 'Cytostatic Factor' (CSF) maintains arrest by preventing loss in Maturation-Promoting factor (MPF) and Emi2 (Early mitotic inhibitor 2) mediated inhibition of Anaphase-Promoting Complex/Cyclostomes (APC/C) (20-24) **Oocyte meiotic II release** Triggers mediated Ca^{2+} oscillations switch on the CaMKII that inactivates Emi2 thereby activating APC and declining MPF activity and release of meiotic arrest.(27-34)	**Mechanical activation** involves vigorous cytoplasmic aspiration before injecting the sperm. **Electrical activation** by generating electric field. **Chemical activation** by using calcium ionophores, strontium chloride (SrCl2), phorbol esters, anhydrous alcohol etc. (39-42)

Conclusion

There have been many mind-boggling questions which remain unanswered despite of continuous cutting-edge research targeting gametic associations especially in the field of assisted reproduction. These questions are often encountered while dealing with repeated failures in IVF/ICSI cycles. Answer to many of these questions is unraveled by concept building and thriving on the newer evolving technologies. Mammalian oocytes undergo cell cycle arrest and resumption by Ca^{2+} homeostasis. The key players in the process are the sperm triggering factors, oocyte machinery constituting molecules of cell cycle and calcium signalling. Many milestones have been attained in understanding the complex phenomenon of oocyte activation but still the search for novel SOAFs is ongoing.

The ART specialists across the globe are dealing with the pressure of ever-increasing burden of failed oocyte activation. Even after having a satisfactory success rates, there are couples who continue to face repeated IVF/ICSI failures which further add on to their emotional burden. Oocyte activation is beyond doubt a complex process and even a seemingly small error in either sperm or oocyte can lead to oocyte activation failure. Hence, there is a need for improving the assisted oocyte activation technologies.

Conflict of interests

Authors declare that there is no conflict of interests.

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