

—Mini Review—

Clinical application of calcium ionophore (A23187) oocyte activation in fertilization failure after ICSI

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Abstract: Fertilization failure occurs in 1–5% of ICSI cases. An abnormality of the oocyte activating ability of spermatozoa is one of a major causes of fertilization failure. For such cases, artificial oocyte activation (AOA) is expected as a method of treating for fertilization failure after ICSI. There are various methods of AOA combined with ICSI. We have adopted treatment with calcium ionophore, A23187, which is a commonly used method, and here, we report on the present status of the methods of oocyte activation combined with ICSI that we use. Although AOA with electrical technique or chemicals stimulation is clinically performed, the safety of this has not yet been proven, and the safer effective methods of AOA are desired.

Key words: Oocyte activation, ICSI, Fertilization failure, Calcium ionophore, A23187

Introduction

Oocytes at the germinal vesicle stage are induced to resume meiosis by the LH surge, and the oocyte maturation arrests again at the metaphase of the second meiotic division (MII). In these process of oocyte activation, fusion of an oocyte plasma membrane and sperm head triggers oocyte activation, and the physiological fertilization leads to increase of inositol 1, 4, 5-trisphosphate (IP_3) concentration in the oocyte cytoplasm. IP_3 stimulates IP_3 receptors on the endoplasmic reticulum (ER), and triggering the release of Ca^{2+} (transient Ca^{2+} increase) from ER. Elevation of the Ca^{2+} concentration in the oocyte cytoplasm leads to activation of the ubiquitin-proteasome pathway and a decreases in the activity of

metaphase-promoting factor (MPF), canceling arrest at MII. Ca^{2+} released from ER is re-absorbed by the ER and released to again to the cytoplasm again. Resulting in transient Ca^{2+} elevations that occurs repeatedly (Ca^{2+} oscillations). The Ca^{2+} oscillations continue for 1 h or more until pronucleus formation, these transient Ca^{2+} increases are important for the resumption of meiosis and embryo development [1, 2].

There are two hypotheses about the mechanism of the increase of IP_3 concentration in the oocyte cytoplasm at the initiation of fertilization. According to the receptor theory, the binding of a sperm to a receptor on the oocyte membrane induces activation of phospholipase C (PLC) via the G-protein/PLC cascade and phosphatidylinositol (4, 5)-bisphosphate (PIP_2) is hydrolyzed to IP_3 by PLC [1]. According to the sperm factor theory, a sperm-derived oocyte activation factor (sperm factor) is released from an immobilized sperm head after intracytoplasmic sperm injection (ICSI) [2]. Sperm factor increases IP_3 , and currently, PLC ζ is thought to be the most likely influential candidate for sperm factor [3]. In the fertilization process, sperm specific PLC ζ flows into an oocyte, and cleaves PIP_2 , and increasing IP_3 in the ooplasm. Then calcium oscillation is induced by the binding of IP_3 to its receptor on ER.

Some studies have reported complete fertilization failure after ICSI occurs in 1–5% [4–8]. It is thought that the causes of fertilization failure after ICSI include disorder of sperm factor, DNA damage in the oocytes and/or spermatozoon, abnormality of the metaphase II spindle, and excessive SS-bonding of the sperm nucleus and so on. Following a spermatozoon injection, 96% of surviving oocytes that undergo spontaneous activation form two pronuclei. When the oocytes with fertilization failure were injected with donated spermatozoa from fertile volunteers as 1day old ICSI, about 70% of them exhibited successfully oocyte activation [9]. Thus, in many cases, fertiliza-

tion failure might be attributable to the spermatozoon side. Therefore, it seems likely that AOA would be useful as measures against fertilization failure after ICSI [9].

Methods of oocyte Activation

It is known that mammalian oocytes can be induced oocyte activation by a variety of chemical and physical stimuli. For the human oocyte, electrical stimulation (electroporation) [10], mechanical activation [11], treatment with calcium ionophore (A231287 [12], ionomycin [13]), treatment with strontium chloride (SrCl_2) [14], PLC ζ [15], puromycin [16] and roscovitine [17] have been reported as the methods of AOA. Puromycin and roscovitine are protein synthesis inhibitors and activate oocytes by inhibiting synthesis of MPF without causing Ca^{2+} increase. Other stimuli induce transient Ca^{2+} rise and activate oocytes.

Mechanisms of AOA

Electrical stimulation (Electroporation)

Electrical stimulation is one of the oocyte activation methods. When a cell is subjected to a continuous current, electrically-charged proteins on the cytomembrane are pulled creating holes in the cytomembrane. Subsequently, extracellular Ca^{2+} inflows into the cytoplasm and the oocyte is activated.

Mechanical activation

When a sperm is injected into an oocyte, repeated aspiration and injection of the cytoplasm by the injection pipette in an oocyte can enhance the entry of Ca^{2+} into the oocyte cytoplasm from outside. In addition, this procedure may also break down endoplasmic reticulum (ER) in the ooplasm, and increasing the concentration of Ca^{2+} in the cytoplasm.

Calcium ionophore

Calcium ionophore is a liposoluble molecule with a high affinity for Ca^{2+} , and neutralizes the positive charge of Ca^{2+} . Calcium ionophore- Ca^{2+} complex can flow into an oocyte cytoplasm against the concentration gradient. A23187 and ionomycin are typical calcium ionophores. Because 1 molecule of Ca^{2+} can bind 2 molecules of A23187 but 1 molecule of ionomycin, the action of ionomycin as a calcium ionophore is stronger than it of A23187.

SrCl_2

Treatment with SrCl_2 is the one of the methods of AOA. Although it is thought that Sr^{2+} , congeneric with Ca^{2+} ,

stimulates IP $_3$ receptor on ER, and Ca^{2+} are released from ER to the oocyte cytoplasm, the details of the mechanism of oocytes activation by Sr^{2+} are unknown. SrCl_2 can induce calcium oscillations in mouse and rat oocytes, but only a transient increase in Ca^{2+} concentration is seen in human oocytes.

PLC ζ

In the process of fertilization, Ca^{2+} oscillations are triggered by PLC ζ carried by a sperm. Thus, PLC ζ injection into an oocytes can induce Ca^{2+} oscillations and activate oocytes.

Puromycin

Puromycin is an inhibitor of protein synthesis. Cyclin B, one of the components of MPF, is blocked its synthesis by puromycin and it reads meiotic resumption. It has been reported that puromycin suppresses the formation of male pronuclei in human oocytes [18].

Roscovitine

Roscovitine inhibits MPF activity selectively. This molecule doesn't inhibit other proteins necessary for fertilization or being, treating oocytes with roscovitine may be safer than treatment with puromycin.

Some pregnancy and delivery cases have been reported in clinical studies involving electro stimulation, A23187, ionomycin and SrCl_2 .

We list the studies in the literature that investigated the documents about clinical studies of the oocyte activation in Table 1. A23187 has frequently been used as AOA with ICSI frequently in the literatures (Table 1). We interpret the treatment of A23187 with ICSI as measure of fertilization failure after conventional ICSI. This clinical study was performed with the approval of the Institutional Review Board and with the informed consent of the couple.

Preparing A23187 Solution

(1) Preparation of stock solution

To prepare 1 mM A23187 stock solution, dissolve A23187 (C7522, SIGMA, U.S.A.) with absolute ethanol, and store at -20°C with protection from light. Prepare new stock solutions once every three months.

(2) Preparing the oocyte activation solution

Mix 10 μl stock solution and 990 μl regular medium for *in vitro* fertilization to prepare 10 μM A23187 solution as an oocyte activation solution. Treat the oocytes with the solution soon after its preparing.

Table 1. Explanation of figures of history of oocyte activation in ICSI fertilization failure cases

| Reference | Indication | Methods of oocyte activation | Cases of pregnancy and delivery |
|---|---|------------------------------|--|
| Tesarik <i>et al.</i> (1994) [12] | fresh oocytes | A23187 | Research |
| Hoshi <i>et al.</i> (1995) [19] | low fertilization | A23187 | 2 cases of pregnancy |
| Yanagida <i>et al.</i> (1999) [10] | fertilization failure | electrical stimulation | 1 case of pregnancy and delivery |
| Yamano <i>et al.</i> (2000) [20] | 1 day old unfertilized oocytes | A23187+ puromycin | Research |
| Nakagawa <i>et al.</i> (2001) [21] | 1 day old unfertilized oocytes | A23187 + puromycin | Research |
| Tesarik <i>et al.</i> (2002) [11] | ICSI patients | mechanical | 1 case of pregnancy and delivery |
| Eldar-Geva <i>et al.</i> (2003) [22] | fertilization failure | A23187 | 1 case of pregnancy and delivery |
| Murase <i>et al.</i> (2004) [23] | fertilization failure | A23187 + puromycin | 1 case of pregnancy and delivery |
| Ebner <i>et al.</i> (2004) [24] | fertilization failure | mechanical | 1 case of pregnancy and delivery |
| Chi <i>et al.</i> (2004) [25] | low fertilization | A23187 | 1 case of pregnancy and delivery |
| Heindryckx <i>et al.</i> (2005) [26] | fertilization failure | A23187 | 3 case of pregnancy in 17 cases |
| Lu <i>et al.</i> (2006) [27] | 1 day old unfertilized oocytes | A23187 + puromycin | Research |
| Manipalviratn <i>et al.</i> (2006) [28] | 1 day old unfertilized oocytes | electrical stimulation | Research |
| Moaz <i>et al.</i> (2006) [13] | low fertilization & fertilization failure | ionomycin | Research |
| Yanagida <i>et al.</i> (2006) [14] | low fertilization | strontium | 1 case of pregnancy and delivery |
| Ahmady <i>et al.</i> (2007) [29] | Immotile testicular sperm | A23187 | 1 case of pregnancy and delivery |
| Nasr-Esfahani <i>et al.</i> (2008) [30] | low fertilization (teratozoospermia) | ionomycin | 2 cases of pregnancy in 87 cases |
| Kyono <i>et al.</i> (2008) [31] | fertilization failure | strontium | 6 cases of pregnancy in 9 cases |
| Heindryckx <i>et al.</i> (2008) [32] | low fertilization | ionomycin | 20 cases of pregnancy in 61 cases |
| Borges <i>et al.</i> (2009) [33] | TESA, PESA sperm | A23187 | 32 cases of pregnancy in 102 cases (Author calculated) |
| Mansour <i>et al.</i> (2009) [34] | fertilization failure, TESE, teratozoospermia | electrical stimulation | 15 cases of pregnancy in 34 cases |
| Terada <i>et al.</i> (2009) [35] | low fertilization | ionomycin | 11 cases of pregnancy and delivery |
| Montag <i>et al.</i> (2012) [36] | low fertilization & fertilization failure | A23187 | 25 cases of pregnancy in 129 cases |
| Vanden Meerschaut <i>et al.</i> (2012) [37] | low fertilization & fertilization failure | ionomycin | 5 cases of pregnancy in 14 cases |
| Ebner <i>et al.</i> (2012) [38] | TESE | A23187 | 29 cases of pregnancy in 75 cases |

Yanagida *et al.* (2008) [16] modified.

(3) Oocyte activation

Incubate the oocytes in the oocyte activating solution for 10 mins in a mixed gas incubator. Then washed them three times and placed them in the regular medium.

Clinical Applications of AOA in ICSI Cycles

We take on four treatment patterns as described below. (Fig. 1, 2). We perform AOA not only for whom we suspect we will only obtain a patients with a history of complete fertilization failure of ICSI in past, but also for patients who might get a few fertilized oocytes (the cases

with previous low fertilization rates in past or a case that rate of second polar body (2ndPb) formation at 4.5–5.0 h after ICSI of $\leq 35\%$).

(1) Rescue oocyte activation (ROA)

When the percentage of the oocytes without a 2ndPb at 4.5–5.0 h after ICSI is more than 65%, we perform ROA for the oocytes without a 2ndPb out of injected oocytes. The cases that receive ROA are recorded as an indication of AOA in the next ICSI cycle.

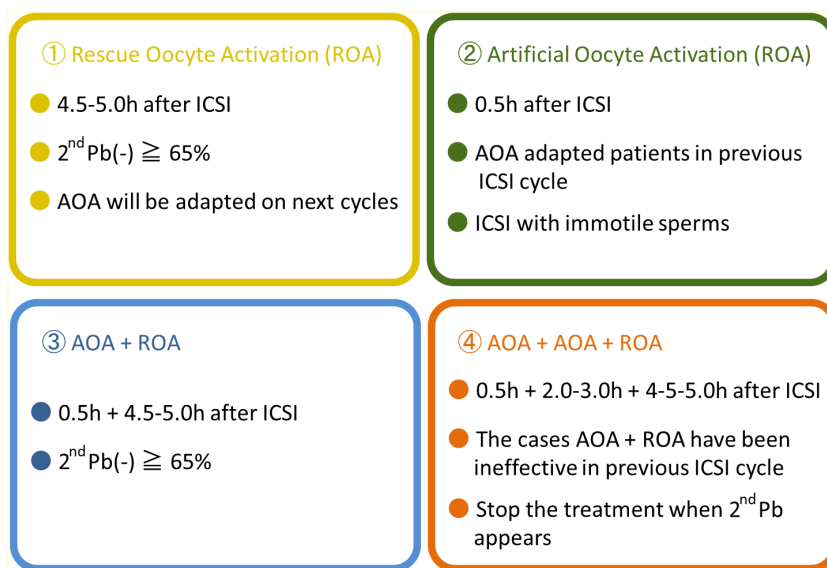


Fig. 1. The treatment patterns of oocyte activation.

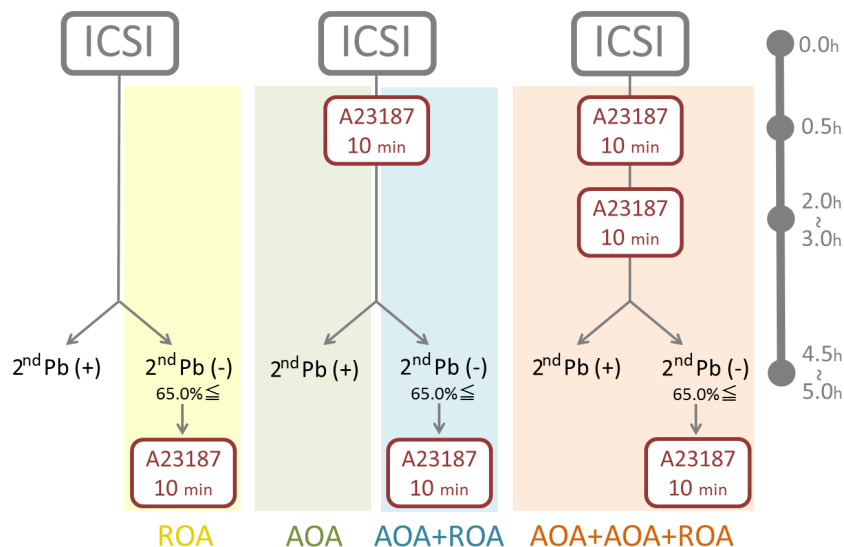


Fig. 2. A flow diagram of the oocyte activation methods combined with ICSI. The right temporal axis shows the time (hour) after ICSI. When the percentage of the oocytes without a second polar body (2ndPb) at 4.5–5.0 hours after ICSI is more than 65%, we performed oocyte activation treatment is performed for the oocytes without a 2ndPb out of injected oocytes.

(2) Assisted oocyte activation (AOA)

A treatment of A23187 is performed 0.5 h after ICSI for the cases with a history of fertilization failure. The fertilization rate of ICSI with immotile spermatozoa is low [39], therefore, we also perform AOA for the cases of ICSI with immotile spermatozoa.

(3) AOA+ROA

We perform oocyte activation twice at 0.5 and 4.5–5.0 h after ICSI for the cases with low fertilization rates in the previous AOA cycle. We consider that the fertilization rate will be low with a single AOA, we perform ROA for the oocytes that are without a 2ndPb at 4.0–4.5 h after AOA.

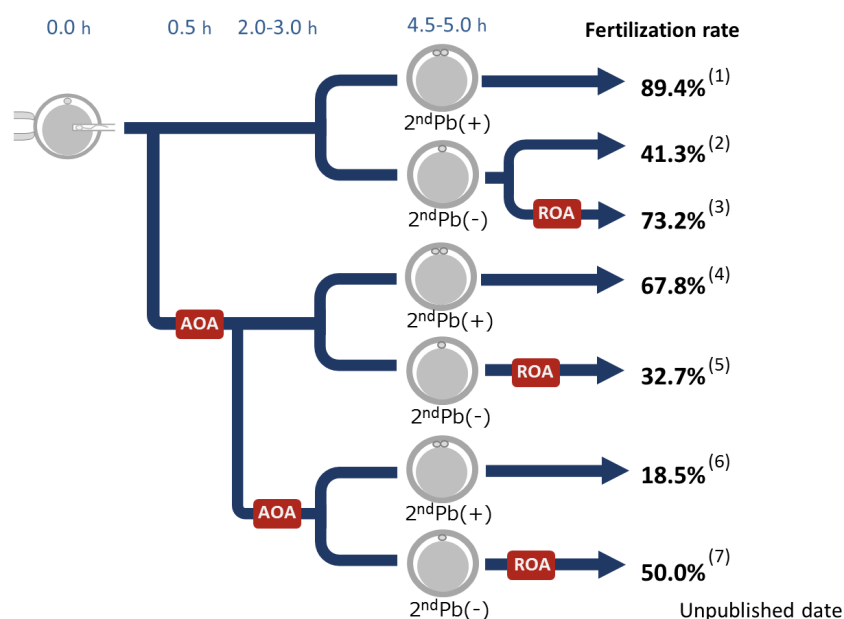


Fig. 3. The outcomes of artificial oocyte activation combined with ICSI. The fertilization rate was evaluated at results in 20 hours after ICSI. (1) Eighty nine percent of oocytes that were recognized a second polar body 4.5–5.0 hours after ICSI were fertilized 20 hours after ICSI. (2) Forty one percent of the oocytes with a second polar body at 4.5–5.0 hours after ICSI were fertilized 20 hours after ICSI. (3) Seventy three percent of the oocytes without a second polar body at 4.5–5.0 hours after ICSI that were continuously treated with ROA were fertilized 20 hours after ICSI. (4) Sixty eight percent of the AOA-oocytes with a second polar body at 4.5–5.0 hours after ICSI were fertilized 20 hours after ICSI. (5) Thirty three percent of the AOA-oocytes without a second polar body 4.5–5.0 hours after ICSI that were treated continuously with ROA were fertilized 20 hours after ICSI. (6) Nineteen percent of the AOA-AOA-oocytes with a second polar body 4.5–5.0 hours after ICSI were fertilized 20 hours after ICSI. (7) Fifty percent of the AOA-AOA-oocytes without a second polar body 4.5–5.0 hours after ICSI that were con.

(4) AOA+AOA (+ROA)

We perform oocyte activation three times at 0.5, 2.5–3.0 and 4.5–5.0 h after ICSI for the cases with a history of a low fertilization rate after AOA+ROA treatment in the past. If a 2ndPb appears before 4.5–5.0 h after ICSI, the third treatment is canceled.

We have been successful at avoiding of fertilization failure by treatment with A23187 as described above. Our treatment outcomes are shown in Fig. 3. The results showed that AOA and ROA were beneficial for the acquisition of the fertilized oocytes.

Safety of ICSI Combined with AOA

Our record of pregnancy/delivery cases in ICSI combined with oocyte activation is presented in Table 1. Up to the present, among the cases we have treated with

AOA, there have been born with a congenital anomaly. Heindryckx reported that calcium ionophore seems not to affect the development of embryos [26]. Kyono reported that there weren't any abnormalities at birth in babies derived from embryos treated with SrCl₂ or A23187, and there were no significant differences in infants' developmental characteristics compared with no activated cases not receiving oocyte activation [40]. There is one follow up study of the babies born after ICSI combined with SrCl₂ oocyte activation together, but it had a small sample size. Meerschaut F.V. *et al.* researched the outcome of development of children who conceived from embryos which have treated with ionomycin for AOA. Their research shows that the neonatal data, cognitive development score and language development scores were within the normal ranges. The studies cited above were conducted with small subject populations, and at

present the accumulated data is insufficient for assessing the safety of AOA yet. [41]

An abnormality of the babies born after oocyte activation has not yet been reported, for the moment. But the data confirming the safety of AOA is scarce at present. Therefore, AOA should be selected, only when it is really necessary.

Conclusion

Treatment of oocytes lacking a 2ndPb with A23187 at 4.5–5.0 h after ICSI is expected to be effective at preventing fertilization failure. In a single treatment case, ROA and AOA are equally effective at avoiding fertilization failure equally. It seems that fertilization failure after AOA might be attributable to the problem in oocytes.

AOA is effective at enabling fertilization in fertilization failure cases and has been widely used in clinical settings. Nevertheless, the oocyte activation methods that are currently used for fertilization failure cases now don't accurately mimic the physiological fertilization process accurately and their safety remains unproven. Therefore, oocyte activation remains an area of the clinical research, rather than an established treatment.

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