

Case Report

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Successful rescue activation of unfertilized oocytes with calcium ionophore (A23187) in a case of recurrent ICSI fertilization failure: A case report

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ABSTRACT

Rationale: This case report describes a couple with recurrent fertilization failure despite undergoing multiple cycles of intracytoplasmic sperm injection (ICSI). The principal clinical concern was suspected oocyte activation deficiency (OAD), in which fertilization is impeded due to the oocyte's inability to initiate embryogenesis, commonly attributed to inadequate intracellular calcium (Ca^{2+}) release following sperm injection.

Patient concerns: The couple repeatedly experienced complete or near-complete fertilization failure in previous ICSI cycles, raising suspicion of an underlying oocyte activation defect.

Diagnosis: Based on the repeated absence of fertilization post-ICSI and clinical history, a diagnosis of suspected OAD leading to recurrent ICSI fertilization failure was considered.

Interventions: Artificial oocyte activation (AOA) using the calcium ionophore A23187 was performed. After ICSI, unfertilized oocytes were exposed to the ionophore to induce Ca^{2+} influx, simulating physiological calcium oscillations essential for oocyte activation. The efficacy of intervention was evaluated through subsequent embryonic development, morphological grading, and chromosomal integrity.

Outcomes: Following AOA treatment, successful oocyte activation occurred, resulting in the formation of high-grade embryos with normal developmental progression. Chromosomal analysis revealed no detectable abnormalities, indicating genomic stability.

Lessons: Calcium ionophore-mediated AOA may serve as an effective adjunct in cases of recurrent ICSI failure attributed to OAD. This case highlights the importance of individualized therapeutic strategies in assisted reproduction; however, further research is needed to refine protocols, validate broader clinical efficacy, and assess long-term safety, including potential epigenetic risks.

KEYWORDS: Oocyte activation deficiency; Intracytoplasmic sperm injection; Artificial oocyte activation; Calcium ionophore A23187; Fertilization failure; Assisted reproductive technology

1. Introduction

The mature oocyte is stimulated to participate in embryo development during fertilization. Depending on the species, various molecular alterations are involved in oocyte activation, usually triggered by the male gamete attaching to the oolemma and inducing intracellular calcium (Ca^{2+}) release during fertilization[1]. Intracytoplasmic sperm injection (ICSI), a widely used assisted reproductive technology (ART), has increased significantly over recent decades—even among normospermic men—rising from 15.4% to 66.9% between 1996 and 2012. Despite typical fertilization rates of 70%–80%, many couples still experience failed or repeated fertilization after ICSI, and evidence also suggests a higher risk of birth abnormalities in ART-conceived children compared with naturally conceived ones[2].

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The significance of Ca^{2+} in oocyte activation (OA) has been demonstrated across species, with research indicating that the pattern of Ca^{2+} release may influence postnatal growth. OA is characterized by wave-like intracellular Ca^{2+} oscillations, primarily mediated by endoplasmic reticulum (ER) Ca^{2+} stores through inositol 1,4,5-trisphosphate (IP3) receptors[3].

To address oocyte fertilization failure, several artificial oocyte activation (AOA) approaches—such as electrical pulse activation, ionomycin, strontium chloride, phospholipase C zeta (PLC ζ), and calcium ionophore (A23187)—have been investigated. Although recombinant PLC ζ mimics physiological oscillations, concerns remain about its impact on embryonic development. Electrical activation creates transient membrane pores allowing Ca^{2+} influx, while calcium ionophore (A23187) has been evaluated in both immediate and rescue activation settings. Recent studies have examined embryo development and single nucleotide polymorphism (SNP) analysis of blastocysts derived from clinically discarded oocytes subjected to calcium ionophore-mediated AOA after fertilization failure[4,5].

This case report describes a patient with multiple ICSI fertilization failures and the successful use of calcium ionophore (A23187) for rescue activation of unfertilized oocytes. The study highlights embryo developmental outcomes and supports personalized approaches to improve ART protocols while emphasizing the need for further evaluation of AOA's long-term safety and efficacy.

2. Case presentation

2.1. Presenting concerns

A 32-year-old female visited the Wardha Test Tube Baby Centre, Acharya Vinoba Bhave Rural Hospital, Wardha, in 2024 for evaluation and management of primary infertility after five years of unsuccessful attempts to conceive. She had undergone two separate ICSI procedures with *in-vitro* fertilization (IVF), both of which were unsuccessful. The patient had a history of regular and predictable menstrual cycles, and no medical issues were detected during her assessment. A semen analysis of her partner, conducted according to the World Health Organization (WHO) 2021 guidelines, revealed standard findings, including a total motile sperm count of 40 million and 50% progressive motility.

2.2. Clinical findings

The patient had been experiencing primary infertility for about five years with no existing medical issues. She underwent her first trial of an IVF-ICSI procedure two years ago, but it ended in complete fertilization failure. A second attempt at IVF-ICSI one year ago also failed to result in a successful pregnancy. Despite having regular ovarian reserve indicators, including an anti-Müllerian hormone

Table 1. Summary of the patient's diagnostic assessment.

Parameters	Value	Reference range
Anti-Müllerian hormone (AMH)	2.8 ng/mL	1.0 – 4.0 ng/mL
Antral follicle count (AFC)	12	10 – 20
Follicle-stimulating hormone (FSH)	6.5 mIU/mL	3 – 10 mIU/mL
Luteinizing hormone (LH)	4.2 mIU/mL	2 – 12 mIU/mL
Estradiol (E_2)	45 pg/mL	25 – 75 pg/mL
Prolactin	12 ng/mL	5 – 25 ng/mL
Thyroid-stimulating hormone (TSH)	1.8 $\mu\text{IU/mL}$	0.4 – 4.0 $\mu\text{IU/mL}$
Semen analysis (Partner)	Normal	WHO 2021 Criteria
Uterine abnormalities (Ultrasound)	None	-
Anti-Müllerian hormone (AMH)	2.8 ng/mL	1.0 – 4.0 ng/mL

level of 2.8 ng/mL and an antral follicle count of 12, fertilization still did not occur. After researching alternative methods to improve fertilization outcomes, the patient chose our facility for her third IVF-ICSI treatment cycle.

2.3. Diagnostic focus and assessment

The patient's baseline hormonal evaluation, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and prolactin, as well as semen analysis, was within normal limits, as shown in Table 1.

2.4. Therapeutic focus and assessment

Ultrasound examination through the vagina revealed that the uterus and ovaries were normal in structure and free of abnormalities. The gonadotropin-releasing hormone antagonist protocol administered during the cycle served as the stimulation technique for controlled ovarian stimulation. The physicians collected 12 oocytes, 5 of which were at the metaphase II (M II) stage. The ICSI procedure was performed, followed by fertilization evaluation 16–18 hours after the injection. However, all oocytes remained unfertilized as shown in Figure 1.

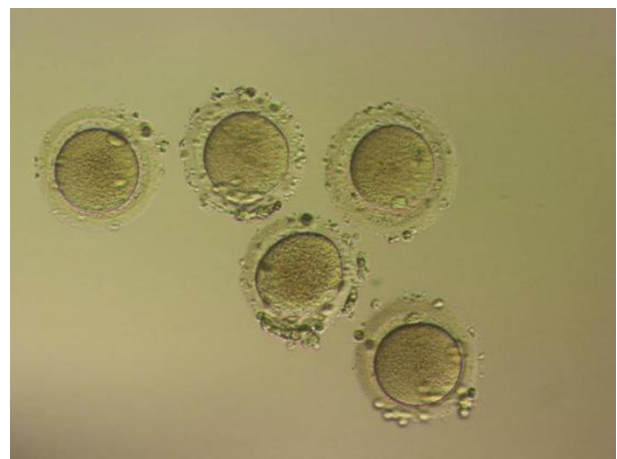


Figure 1. Unfertilized metaphase II oocytes from a 32-year-old woman with primary infertility, showing complete fertilization failure after intracytoplasmic sperm injection (ICSI).

To address this, the oocyte rescue activation procedure involved applying the calcium ionophore A23187. The researchers placed the unfertilized oocytes in a medium containing calcium ionophore for 15 minutes before returning them to standard embryo culture conditions. After 6 hours, fertilization assessment revealed that six out of ten activated oocytes successfully formed two pronuclei (2PN). These fertilized oocytes proceeded to embryo development, with four reaching the blastocyst stage by Day 5. Two high-grade blastocysts were cryopreserved for potential future embryo transfer.

2.5. Follow up

The patient underwent frozen embryo transfer treatment combined with estrogen and progesterone for endometrial preparation. The ultrasound-guided embryo transfer involved a single blastocyst, and luteal phase support was administered simultaneously. A β -human chorionic gonadotropin (β -hCG) test, conducted with serum samples taken 12 days after the transfer, showed a result of 350 mIU/mL. A 6-week pregnancy check *via* ultrasound revealed a normal placental position and a detectable fetal heartbeat. Implementing calcium ionophore A23187 rescue activation yielded successful results during ICSI treatment for a patient who had experienced repeated fertilization failure.

2.6. Ethics statement

The study was approved by Datta Meghe Institute of Higher Education and Research [DMIHER(DU)/IEC/2024/178; 31/01/2024]. Consent was obtained from the patient prior to the procedure.

3. Discussion

Miao and Williams demonstrated the importance of spatiotemporal regulation in Ca^{2+} signal generation and transduction, noting that advances in genetically encoded Ca^{2+} sensors and high-resolution microscopy will further accelerate this field[6]. Zhang *et al* reported that calcium ionophore (A23187) activation improved chromosomal integrity in blastocysts, reduced ICSI fertilization failure, and did not negatively affect early embryo development, aneuploidy rates, birth weight, or gestational age[4].

Ruan *et al* suggested that ionomycin-based AOA can improve reproductive outcomes in patients with poor embryo development or a history of prior fertilization failure. Subgroup analyses indicated potential benefits across male, female, and unexplained infertility factors; however, broader studies are needed to confirm safety, efficacy, and more physiological activation methods[7].

Tejera *et al* highlighted the need to evaluate PLC ζ levels in patients

with impaired fertility and found that AOA increased the number of surplus embryos, frozen embryos, implantation rates, and ongoing pregnancy rates, supporting the need for further validation of AOA's effectiveness[8].

Swann emphasized the role of Ca^{2+} oscillations in human zygotes during ICSI, demonstrating that PLC ζ -induced Ca^{2+} transients produce subtle cytoplasmic movements, offering a minimally invasive method to quantify Ca^{2+} spikes during fertilization[9].

In conclusion, this case demonstrates successful rescue activation of unfertilized oocytes using calcium ionophore (A23187) in a patient with repeated ICSI fertilization failure. Calcium ionophore facilitated oocyte activation, supported normal embryo development, and resulted in a confirmed pregnancy. This case reinforces the selective use of AOA in confirmed oocyte activation deficiency and underscores the need for standardized protocols and long-term safety evaluation.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Authors' contributions

Akash More, Vilas Chimurkar, Namrata Choudhary, Dipali More, and Sanket Mahajan were involved in clinical evaluation, patient management, and collection of relevant clinical data. They contributed to the interpretation of findings and drafting of the case report. All authors participated in critically revising the manuscript for its intellectual content. All authors approved the final version of the manuscript for publication.

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References

[1] Neri QV, Lee B, Rosenwaks Z, Machaca K, Palermo GD. Understanding

- fertilization through intracytoplasmic sperm injection (ICSI). *Cell Calcium* 2014; **55**(1): 24-37. doi: 10.1016/j.ceca.2013.10.006.
- [2] Kashir J, Ganesh D, Jones C, Coward K. Oocyte activation deficiency and assisted oocyte activation: Mechanisms, obstacles and prospects for clinical application. *Hum Reprod Open* 2022; **2022**(2): hoac003. doi: 10.1093/hropen/hoac003.
- [3] Abdulsamad HMR, Murtaza ZF, AlMuhairi HM, Bafleh WS, AlMansoori SA, AlQubaisi SA, et al. The therapeutic and diagnostic potential of phospholipase C zeta, oocyte activation, and calcium in treating human infertility. *Pharmaceuticals (Basel)* 2023; **16**(3): 441. doi: 10.3390/ph16030441.
- [4] Zhang J, Yao G, Zhang T, Hu J, Yang G, He J, et al. Effect of calcium ionophore (A23187) on embryo development and its safety in PGT cycles. *Front Endocrinol (Lausanne)* 2022; **13**: 979248. doi: 10.3389/fendo.2022.979248.
- [5] Xu Z, Yao G, Niu W, Fan H, Ma X, Shi S, et al. Calcium ionophore (A23187) rescues the activation of unfertilized oocytes after intracytoplasmic sperm injection and chromosome analysis of blastocyst after activation. *Front Endocrinol (Lausanne)* 2021; **12**: 692082. doi: 10.3389/fendo.2021.692082.
- [6] Miao YL, Williams CJ. Calcium signaling in mammalian egg activation and embryo development: The influence of subcellular localization. *Mol Reprod Dev* 2012; **79**(11): 742-756. doi: 10.1002/mrd.22078.
- [7] Ruan JL, Liang SS, Pan JP, Chen ZQ, Teng XM. Artificial oocyte activation with Ca^{2+} ionophore improves reproductive outcomes in patients with fertilization failure and poor embryo development in previous ICSI cycles. *Front Endocrinol (Lausanne)* 2023; **14**: 1244507. doi: 10.3389/fendo.2023.1244507.
- [8] Tejera A, Alegre Ferri L, Gamiz Izquierdo P, Beltrán Torregrosa D, Alejandro Remohí J, Meseguer Escrivá M. Treatment with calcium ionophore improves the results in patients with previous unsuccessful attempts at the fertilization: A cohort study. *Int J Fertil Steril* 2021; **15**(4): 286-293. doi: 10.22074/ijfs.2021.136168.1013.
- [9] Swann K. The role of Ca^{2+} in oocyte activation during *in vitro* fertilization: Insights into potential therapies for rescuing failed fertilization. *Biochim Biophys Acta Mol Cell Res* 2018; **1865**(11 Pt B): 1830-1837. doi: 10.1016/j.bbamcr.2018.05.003.