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Case Report

First Reported Application of Calcium Ionophore at the Human Cleavage Stage: A Case Report

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Abstract

Background: *Calcium ionophore (A23187) is commonly used in assisted reproductive technology, where it has shown significant potential to enhance fertilization rates and embryo development. It facilitates the movement of calcium ions across cell membranes, thereby manipulating the intracellular calcium signaling in sperm and oocytes. The efficiency and safety of calcium ionophore (A23187) activation, its effects on embryo developmental potential, and its effects on pregnancy outcomes after embryo transfer have been approved.*

Case: *A 32-year-old woman was first seen at our hospital in January 2025. She presented with a history of secondary infertility for the past 8 months and has no living children. She experienced three spontaneous pregnancies and one pregnancy following ovarian stimulation, but all resulted in chemical pregnancies. Her hormonal profile indicated a polycystic ovary case with normal FSH, LH, and Prolactin.*

Conclusion: *Our findings demonstrate that Calcium Ionophore (A23187) activation is a safe and effective strategy for enhancing blastocyst formation and euploidy embryos.*

Keywords: *Calcium Ionophore (A23187); ICSI; AOA; cleavage stage embryos; blastocysts); Euploidy.*

Introduction

In contrast, calcium ionophore (A23187) and ionomycin can activate oocytes by enhancing the cell membrane's permeability to calcium ions. In 1995, the first successful birth of a baby using Intracytoplasmic Sperm Injection (ICSI) combined with calcium ionophore (A23187) activation was reported. (1)

Calcium ionophore (A23187) is a highly selective calcium ion carrier frequently used in artificial oocyte activation (AOA). A meta-analysis showed that calcium ionophore (A23187) helps promote oocyte activation and improves fertilization, blastocyst formation, embryo implantation, and live birth rates. (2)

Studies indicate that calcium ionophore (A23187) helps rescue fertilization in cases of ICSI fertilization failure by activating oocytes and improving fertilization and cleavage rates without increasing the risk of meiotic errors or affecting embryo development timing. (3)

Not all embryos develop into blastocysts. It is well known that late paternal effect and embryonic genome activation occur around day 3 of embryo development. (4) Currently, no research examines the impact of calcium ionophore (A20-3187) activation on embryonic aneuploidy during the human preimplantation genetic testing (PGT) cycle. Therefore, it is important to investigate in detail the potential gene expression and epigenetic changes that may result from calcium ionophore (A23187) activation.

Patient Presentation:

Background: A 32-year-old female with a history of secondary infertility for eight months presented to Al Baraka Fertility Hospital for evaluation after experiencing four spontaneous pregnancies, all chemical pregnancies, and one failed intrauterine insemination IUI.

Clinical History:

The patient's infertility workup revealed no significant anatomical or hormonal abnormalities, and has been actively trying to conceive, with a focus on optimizing her chances through IVF. Recent Intervention: Routine investigations for unexplained infertility revealed the following results: the anti-Mullerian hormone (AMH) level was 2.49 ng/ml, and the endometrium appeared clear. Two egg collection cycles were performed, and the results.

Results

Developmental Stage	FIRST (NO Ca++)	2nd (with Ca++)	P-Value
Eggs Retrieved	34	49	(Baseline)
Maturation Rate (M2)	30 / 34 (88.2%)	38 / 49 (77.6%)	0.204
Fertilization Rate (2PN)	26 / 30 (86.7%)	33 / 38 (86.8%)	0.984
D2 Cleavage (4 Cells)	26 / 26 (100%)	33 / 33 (100%)	N/A
D3 Cleavage (8 Cells)	18 / 26 (69.2%)	26 / 33 (78.8%)	0.395
Blastocyst Formation Rate	12 / 26 (46.2%) *	21 / 33 (63.6%) *	0.173
Euploid Rate (per Blastocyst)	5 / 12 (41.7%)	10 / 21 (47.6%)	0.736

The maturation rates for these cycles were 88.2% vs 77.6% while the fertilization rates were 86.8% vs 86.8% and at D2, all fertilized oocytes were cleaved in both groups.

Calcium ionophore was applied for the 2nd trial embryos at D2 (4-cell stage), and both groups were checked at D3 to confirm the improvement of embryos from D2 to D3, and the results showed 69.2% for the first group vs 78.8% for the 2nd group. However, the blastulation rates, which were recorded at 46.2% vs 63.6. On the other hand, the euploidy rate is 41.7% vs 47.6%.

Discussion

Calcium ionophore (A23187) is commonly utilized in clinical practice for AOA due to its ease of use and accessibility. Studies have shown that calcium shock plays a major role in successfully inducing oocyte activation (5). A systematic review suggested that AOA with calcium ionophore treatment after intracytoplasmic sperm injection (ICSI) results in a statistically significant improvement in fertilization, cleavage, and blastulation, demonstrating that the strong effect of calcium ionophore use is reassuring and promising (6).

It is important to point out that using calcium ionophore (A23187) does not increase the risk of fetal aneuploidy, preterm delivery, or low birth weight (7,8). Is calcium's control of mitotic progression unique to fertilization or does it represent a common mechanism across all cell cycles? This is the question. Right after fertilization, embryos begin a sequence of cell divisions. The embryos of species that use external fertilization exhibit a specific speed-up in this process. On Day 2, embryos are at the four-cell stage of development by 45.5–45.7 hours post sperm insemination, and three days after fertilization are at the eight-cell stage by 54.3–56.4 hours (9,10).

However, a systematic study of calcium and cleavage has yet to be conducted. Indeed, earlier work in mammalian embryos could not detect cleavage-related calcium signals.

Calcium fluctuations were observed with a peak just before cell division. Interestingly, these calcium oscillations were absent in human embryos that were arrested. Mitotic division blocked with a Ca (2+) chelator could be restored through ionophores in animal models (11).

Any significant disruption in calcium homeostasis will directly and definitively lead to the failure of both compaction and blastocyst formation, resulting in embryonic arrest, which is largely accurate. Calcium signaling is critical for normal embryonic development, and severe disruptions can cause developmental problems or arrest (12). Calcium is essential for cell division and proliferation and affects various stages of the cell cycle. Calcium transients, observed during the late G1 phase and mitosis, are mainly caused by the release and reuptake of calcium by the endoplasmic reticulum (13).

Recent research has provided the role of calcium in mitosis, particularly in chromosome segregation and the prevention of aneuploidy. Strong evidence indicates that calcium signals are essential for regulating cell-cycle transitions in rapidly dividing embryos (14).

There is strong literature support for the use of calcium ionophores to increase intracellular calcium levels in embryos, particularly in cycles with poor embryo growth and blastocyst formation, as a strategy to avoid transfer cancellation. Studies have shown that artificial oocyte activation (AOA) with ionophores such as A23187 or ionomycin can improve embryonic development rates and sometimes clinical pregnancy chances in cases with previous poor outcomes after ICS. (15)

It is essential to note that calcium fluctuations were detected with a peak shortly before cell division. Interestingly, these calcium oscillations disappeared in arrested embryos (16)

Conclusion

This is the first report to mention Calcium Ionophore (A23187) on Cleavage Stage Embryos at D2 (4 cells). In D2 embryos (4-cell stage embryos), calcium ionophore (A23187) may increase the number of blastocyst embryos at D5 or D6 and number of euploidy rate. More research is needed to improve the use of Calcium Ionophore (A23187) with cleavage cell embryos.

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Conflict of interest

The authors would like to state that they have no competing interests to disclose.

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