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Double Ionophore Activation (DIA) Technique In Cases Of Severe Male Factor: Case Series

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Abstract

Background: Sperm binding or injection into human oocytes induces calcium oscillations resulting in oocyte activation, fertilization, and subsequent embryonic development. In severe male factor, sperm may fail to activate oocytes with subsequent ICSI failure. Artificial oocyte activation with calcium ionophore can induce calcium influx with improved oocyte activation and development.

Objective: The current case series evaluated a unique technique of double ionophore activation (DIA) in severe male factor without previous ICSI failure.

Materials and Methods: Case series of 17 infertile couples with severe male factors undergoing ICSI. The injected oocytes were incubated in calcium ionophore twice with this new protocol for artificial oocyte activation.

Results: Fertilization, cleavage, and blastocyst rates were 71.4%, 100%, and 73.3% respectively. The pregnancy rate for fresh transfer was 9/13 (69.2%) and the pregnancy rate for FET was 4/6 (66.7%).

Conclusion: DIA protocol may improve oocyte activation in severe male factor and a well-designed prospective study is needed.

Keywords: Oocyte activation, oligoteratozoospermia, calcium ionophore, fertilization, ICSI, Blastocyst rate.

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Case Series

Introduction

Oocyte activation results in the resumption of meiosis II (MII), second polar body (2PB) extrusion, cortical granule exocytosis, and cytoskeletal rearrangements (1- 2).

Sperm oocyte attachment and fusion trigger intra-ooplasmic calcium (Ca^{2+}) oscillations, essential for releasing oocytes from meiotic arrest and triggering the embryonic development program (3-5).

The precise mechanisms underlying Ca^{2+} oscillations in mammals have been subject to much debate, particularly regarding the roles played by both gametes. Three predominant models were hypothesized: (i) the Ca^{2+} conduit model (6), (ii) the membrane receptor model (7), and (iii) the soluble sperm factor model (8). However, significant evidence supports the theory of sperm factor mediation within mammals (9).

In the "soluble sperm factor model," entry of the spermatozoon releases a sperm oocyte activating factor ((SOAF), phospholipase C zeta (PLCζ) protein or post acrosomal WW domain-binding protein (PAWP) that hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) (10- 11). Afterward, IP3 binds to specific receptors on the endoplasmic reticulum that induce further Ca^{2+} oscillations, resulting in oocyte activation (12). Ca^{2+} signals are also essential for the control of the subsequent embryo divisions (13). The number of calcium oscillations increases as the physiologic processes proceed from cortical granule exocytosis through alleviation of MII arrest (14), eventually reaching complete oocyte activation, requiring the highest number of oscillations (15). The amplitude, frequency, and duration of Ca^{2+} oscillations constitute signals for the embryo's developmental potency and considerably affect the pre- and post-implantation period (16).

The absence of the phospholipase C zeta protein ((SOAF) in the sperm head is associated with direct failure to signal Ca^{2+} oscillations (10).

Calcium surge resulting in artificial oocyte activation (AOA) can be induced from two potential Ca^{2+} sources: internal calcium storages and/or external culture medium (17).

Endoplasmic reticulum (ER) is the main source of Ca^{2+} release at fertilization, and extracellular Ca^{2+} influx is required to maintain these Ca^{2+} oscillations, a process known as calcium-induced calcium release (CICR) (18). Depletion of extracellular Ca^{2+} reduces the frequency of or completely ceases Ca^{2+} oscillations (19). Physiologic CICR activation is acquired during oocyte maturation (20). Calcium stores are regulated by a mechanism termed store-operated Ca^{2+} entry (SOCE), which maintains intracellular Ca^{2+} homeostasis from the extracellular milieu (21). Larger stores, more channels, and reorganization of ER increase calcium sensitivity with more calcium release (22). The response from 'fertile' males is extremely variable, with 20% of human sperm exhibiting only 1 or 2 Ca^{2+} transients upon injection into oocytes (23), which is unlikely to activate oocytes (24). Furthermore, 10% of such sperm do not elicit any Ca^{2+} release at all, suggesting that the ability of normal human sperm to cause Ca^{2+} signals is likely to be highly variable; this is in accordance with observations of variable localization patterns and levels of PLC zeta in human sperm and may underlie cases of low fertilization success (25).

Natural oocyte activators include phospholipase C zeta (26,27), and WW-binding domain protein' (PAWP) (28).

Men with oocyte activation deficiency (OAD) showed lower percentages of sperm cells expressing PLCζ and PAWP and altered localization of PLCζ compared to fertile men (29). PLCζ and PAWP are two important sperm oocyte activating factors (SOAFs), and their impairments may be considered as one of the possible causes of subfertility in OAD.

Methods of artificial oocyte activation include mechanical methods, which involve oolema penetration by micromanipulation, followed by strong cytoplasmic aspiration, inducing a Ca^{2+}

influx (30). Mechanical activation usually results in a single Ca^{2+} increase (31).
Chemical activation is done by Ca^{2+} ionophores, such as ionomycin and Ca^{2+} ionophore A23187, which increase Ca^{2+} permeability of the cell membrane, allowing extracellular Ca^{2+} to flow into the oocytes and provoke intracellular Ca^{2+} stores to release stored Ca^{2+} (32). However, neither Ca^{+2} ionophores can produce Ca^{2+} oscillations (33). Other activating agents have been shown to cause multiple transients and include strontium chloride ($SrCl_2$), which is more successful in producing multiple oscillations, better fertilization, and embryo development (32, 34).

Combinations of chemical activators could result in a higher blastocyst rate (35) with fewer chromosomal abnormalities (36). ICSI-AOA increased fertilization rates in infertile couples with teratozoospermia as well (37). Activating factors did not cause post-implantation malformations in the offspring (32). They did not increase imprinting disorders in live births as well (38).

In a recent small-size study, AOA changed imprinting methylation rates at certain genes in cleavage embryos but not in the blastocyst stage and placenta. It was recommended that blastocyst transfer should be considered for patients undergoing AOA during in vitro fertilization (39). This is further supported by the findings of a recent article showing that Calcium ionophore (A23187) activation did not affect the proportion of early embryonic development and blastocyst aneuploidy; furthermore, it did not affect the gestational age and weight at birth (40).

Aim of the work

Evaluate the effect of double application of calcium ionophore for AOA in couples with severe oligoasthenospermia.

Patients

Case series of 17 Infertile couples with severe oligoasthenospermia. Sperm count <5 and motility <15% scheduled for ICSI. Setting: Dar Elteb IVF Center, Alexandria, from January 2023 to October 2023. All oocytes were frozen in 6 patients, and 11 had fresh transfer.

Methods

Ovarian stimulation protocol as described before OPU was performed 37 hours after hCG. The time of oocyte denudation was adjusted to 38 hours after hCG (41).

Calcium ionophore preparation: One tube of calcium ionophore stock solution (Sigma Aldrich, UK) was warmed at room temperature. Add 5 microns of stock solution to 995 microns of global media (Cooper Surgical Fertility Solution, Denmark) to make a concentration of 1ml calcium ionophore working solution.

Double Ionophore Activation (DIA) Protocol:

First calcium ionophore activation: After all oocytes were injected, they were immediately incubated in calcium ionophore working solution for 15 minutes, washed four times in embryo culture medium, and then incubated to rest in culture media for 30 minutes.

Second calcium ionophore activation: oocytes were incubated again in calcium ionophore for 15 minutes, washed 4 times in embryo culture media, and then transferred into final incubation dishes. Check for fertilization and cleavage were done as usual. Embryo transfer (ET) was done on day 5, with a maximum of 2 blastocysts transferred. hCG was checked on Day 15 after ET. Ultrasound to detect clinical pregnancy at day 22 after hCG.

Results

The median age of the studied women (n=17) was 33 years, 95% CI (31.00-37.00). Sperm count/ml ranged from < 1 million to 5 million/ml. Total sperm motility ranged from <5% to 10%. The median number of M2 oocytes, zygotes, cleaved embryos, and blastocysts were 11, 7, 6, and 5, respectively (table 1). Regarding IVF laboratory key performance indices (KPIs), fertilization, cleavage, and blastocyst rates were 71.4%, 100%, and 73.3% respectively (table 2). Fresh transfer was performed in 11 patients with seven clinical pregnancies

(63.6%). Frozen embryo transfer (FET) was done in 6 cycles, 4 of which resulted in clinical pregnancy (66.7%) Table 3.

Table 1: Number of oocytes, zygotes, embryos, and class A embryos

Total oocytes	
Min-Max	3.00-27.00
Median	12.00
95% CI of the median	9.00-18.00
25th Percentile – 75th Percentile	8.00-17.00
Total M2	
Min-Max	3.00-18.00
Median	11.00
95% CI of the median	8.00-16.00
25th Percentile – 75th Percentile	7.00-14.00
Fertilized	
Min-Max	3.00-15.00
Median	7.00
95% CI of the median	7.00-11.00
25th Percentile – 75th Percentile	5.00-10.00
Cleaved	
Min-Max	2.00-15.00
Median	6.00
95% CI of the median	5.00-11.00
25th Percentile – 75th Percentile	5.00-10.00
Total number of Embryos Class A	
Min-Max	2.00-11.00
Median	5.00
95% CI of the median	5.00-11.00
25th Percentile – 75th Percentile	4.00-5.00

Min-Max: Minimum – Maximum
CI: Confidence interval

Discussion

Ebner et al., in an earlier study, showed that ready-made Ca^{2+} ionophore resulted in a 57% fertilization rate, 56% blastocyst rate, 29.7% Clinical pregnancy, and 34.2% live birth rates in couples with azoospermia and cryptozoospermia with previous unsuccessful cycles (42). In another publication, using the same technique of ready-to-use ionophore, they reported 47% fertilization, 57% blastocyst, and 37% clinical pregnancy rate in couples with previous fertilization problems (43). In the current preliminary study, oocytes were exposed twice to double ionophore activation

Table 2: Fertilization, cleavage, and blastocyst rates

Fertilization rate (%)	
n	17
Min-Max	57.10-100.00
Median	71.40
95% CI of the median	71.40-87.50
25th Percentile – 75th Percentile	64.70-83.30
Cleavage rate (%)	
n	17
Min-Max	50.00-100.00
Median	100.00
95% CI of the median	100.00-100.00
25th Percentile – 75th Percentile	85.70-100.00
Blastocyst rate (%)	
n	17
Min-Max	45.45-100.00
Median	73.30
95% CI of the median	62.50-100.00
25th Percentile – 75th Percentile	60.00-100.00

n: Number of patients
Min-Max: Minimum – Maximum
CI: Confidence interval

Table (3): Pregnancy rate in fresh and frozen cycles

Pregnancy rate (Fresh transfer)	
n	7/11
%	63.6%
95% CI	35.68-91.914%
Pregnancy rate (FET)	
n	4/6
%	66.7%
95% CI	39.58-98.95%

n: Number of cycles
Min-Max: Minimum – Maximum
CI: Confidence interval

(DIA). As calcium ionophore produces a single spike (rather than calcium oscillations (33), we hypothesized that DIA could be more physiologic. In this very limited sample size, fertilization 71%, blastocyst 73%, and pregnancy rate 78.6% were encouraging. Unlike previous studies, couples involved did not experience previous fertilization problems.

A meta-analysis reported that calcium ionophore increased LBR significantly (44). In subgroup analysis, calcium ionophore positive effect was mainly in patients with previous low fertilization, fertilization failure, and cleavage problems. ESHRE Recommended that artificial

oocyte activation using Ca^{2+}-ionophores is mainly recommended for cases of complete activation failure (0% 2PN), and very low fertilization (17). In the current preliminary report, such a positive effect was also obtained in couples without a history of impaired fertilization.

Conclusion

Case series indeed represent the bottom of the evidence hierarchy, but they can point out ideas that need further research to prove or refute the findings. Regarding the current case series, there is a need to evaluate double ionophore activation with a good design and adequate sample size.

In conclusion, a well-designed prospective study is needed to evaluate ICSI performance and pregnancy rate of DIA in severe male factor.

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