

Assisted Oocyte Activation Method (Ca ACTIVATION) Improves Fertilization in Patients with Recurrent Fertilization Failure

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Abstract: Total fertilization failure (TFF) happens in 1–3% of total intracytoplasmic sperm injection (ICSI) cycles and may reoccur in sequent cycles. Regardless the high success rate with the applying of intracytoplasmic sperm injection (ICSI), there's still a little number of couples who cannot get fertilized eggs after using ICSI. Six couples experiencing recurrent TFF or low fertilization rate (<10%) after ICSI were recorded in this study. Compared with the regular ICSI group and the conventional ICSI-AOA group, the new AOA method using calcium ionophore, can seriously increase the fertilization rate from less than 10% up to approximately 50% in most cases. The normal distribution of sperm-related oocyte activation factor phospholipase C zeta (PLCz1) in the sperms of the cases indicated the absence of an aberrant Ca signaling activation.

AOA method using calcium (Calcium ionophore A23187) was able to effectively improve the fertilization rate in the majority of patients suffering from TFF. This AOA method had a potential therapeutic effect on those couples experiencing TFF, even after intracytoplasmic sperm injection (ICSI), which may overcome the severe fertilization deficiencies in patients with a repeated low fertilization or TFF.

Keywords: total fertilization failure, Calcium ionophore A23187, fertilization, assisted oocyte activation.

I. INTRODUCTION

Assisted reproductive techniques (ART) provide the infertile couples the chance to conceive, and also the advance of intracytoplasmic sperm injection (ICSI), a method involving the injection of one sperm into the protoplasm of a mature oocyte, has allowed the accomplishment of pregnancy for couples affected by severe male factor infertility, low fertilization rates after standard in vitro fertilization (IVF), or unexplained infertility. Though nowadays, a fertilization rate of roughly 70% is determined during ICSI cycles with a clinical pregnancy rate of 45%, total fertilization failure (TFF) still

Happens in 1–3% of the overall ICSI cycles and might reoccur in following cycles, even once enough numbers of mature oocytes are available (Nasr-Esfahani et al., 2010). TFF is usually a physical misery and emotional stress for sterile couples.

Therefore, it looks to be considerably vital to explore the reasons and get the solutions of TFF.

There is currently a general agreement that oocyte activation deficiency should be principally responsible for TFF following standard ICSI (Vanden Meerschaut et al., 2014b). Many studies have shown that the bulk of couples affected by TFF can have the benefit of assisted oocyte activation (AOA) following ICSI (Sfontouris et al., 2015; Bonte et al., 2019), and in some centers, AOA is sometimes offered to couples experiencing TFF or a really low fertilization rate after being well advised. The well-established and most typically represented AOA protocols will be classified into 3 totally different methods, i.e., mechanical, electrical, and chemical stimuli (Nasr-Esfahani et al., 2010).

Calcium ionophore, as well as ionomycin and A23187 (also referred to as calcimycin), is one of the foremost Widely used AOA agents within the human ART process. It aims to raise Calcium artificially in oocyte cytoplasm for Calcium oscillations and might either promote Calcium flow from the extracellular medium by altering the cellular membrane permeability or target directly on the endoplasmic reticulum (ER) to release Ca²⁺ (Swann, 2018). Despite the high success rate with the applying of AOA, there's still atiny low range of couples who cannot get fertilized eggs when standard ICSI-AOA used (Vanden Meerschaut et al., 2012).

It is usually accepted that oocyte activation process could be a complicated and spatial-temporal regulated Method begin by the entry of the sperm cell. Several minutes after sperm-oocyte cell membrane interaction, intracellular Calcium oscillations flowing from the ER are elicited by the discharge of a soluble sperm factor named phospholipase C zeta (PLCz). A series of calcium-sensitive downstream pathways are then activated by these Calcium changes, more additional the inactivation of the maturation promoting factor (MPF), the block of which conversely contributes to the prevalence of the early events of oocyte activation, like the restart of meiosis and also the discharge of the second polar body (Kashir et al., 2013; Vanden Meerschaut et al., 2014b). oocyte activation is a series of calcium-sensitive reactions followed by MPF inactivation, and conventional ICSI-AOA is applicable for the majority of TFF, that was mainly refer to the shortage of Calcium oscillations.

2. MATERIALS &METHODS

Total of six couples undergoing infertility treatment by ART within the

Reproductive Center, EL nada IVF HOSPITAL, between nov2020 and Dec 2021 were listed within the study.

Those couples experienced recurrent unsuccessful or low fertilization (<10%) after ICSI, that is not a satisfied results

Seminal fluid Analysis and Preparation

Fresh ejaculated semen was obtained by masturbation and picked up on the oocyte retrieval day in sterile containers and kept for about an hour at 37°C for liquefaction followed by concentration, total motility, viability, and morphology analyses.

The seminal fluid analysis was supported the standard of the fifth edition of the WHO guidelines.

A normal semen sample must to be equipped with a minimum of a concentration of 15 × 10⁶ /ml, a complete motility of 40%, and a standard morphology rate of 4% (Xin et al., 2020).

Standard density- gradient centrifugation was performed for sperm cell choice as previously represented (Huang et al., 2015). Briefly, 1.5 ml of 45%

gradient media (Vitrolife, Gothenburg, Sweden) was added on the highest of 1.5 mL of 90% gradient media.

Then, up to 3 ml of the semen was layered on the gradient media and centrifuged at 200 g at room temperature for 20 min.

The sperm pellet was isolated and washed with 3 ml of sperm Washing Medium (Vitrolife, Gothenburg, Sweden) at 300 g for 6 min.

Then, the washing pellet was resuspended in 0.5 ml of sperm cell washing Medium and left at room temperature to permit for a swim- up for 30–60 min.

The top the 300 m L was collected for semen parameters analysis.

Table 1 Semen parameters of the participants involved.

	Concentration of (×10 ⁶ /mL)	Total motility(%)	Normal morphology rate (%)	Diagnosis*
Case 1	6.19	26.00	9.00	Oligopermia
Case 2	50.11	41.12	12.00	normal
Case 3	47.51	42.40	10.00	normal
Case 4	46.66	70.70	2.50	teratozoospermia
Case 5	31.10	47.70	3.77	teratozoospermia
Case 6	121.00	65.60	11.00	normal

Note* :The seminal fluid analysis was supported the standard of the fifth edition of the who guidelines. a standard semen sample should be equipped with a minimum of amount of 15×10⁶/ml, a complete motility of 40%, and a standard morphology rate of 4%.

Oocyte Retrieval and Fertilization

All participants undergo a controlled ovarian stimulation that was processed based on the previous study (Wang et al., 2021.)

Oocytes were retrieved by transvaginal ultrasound 36–38 h once human chorionic gonadotropin administration.

For ICSI patients, cumulus- oocyte complexes were exposed to 80 U/L hyaluronidase (Irvine Scientific, United States) followed by mechanical pipetting for

Degranulation. Nude oocytes were any cultured for one more 1-2 h before sperm injection.

Conventional ICSI-AOA Procedure

Conventional ICSI-AOA procedure was performed as previously represented (Capalbo et al., 2016). Briefly, freshly collected MII oocytes were exposed to pre-equilibrated Ca-ionophore A23187 (GM508 Cult Active, Gynemed, Germany) for 15 min once the regular sperm injection.

Fertilization was assessed 18 h after ICSI, and traditional fertilization was characterized by 2 distinct pronuclei and 2 polar bodies. Embryo quality was evaluated fertilization. Statistical Analyses

All data were analyzed using the Statistical Package for the Social Sciences (SPSS 22.0, IBM, Armonk, NY, United States). The data were presented as mean ± standard deviation (SD) for continuous variables and percentages for categorical variables. The chi-square test and Fisher’s exact test were performed for continuous variables.

The Kruskal-Wallis non-parametric rank-sum test was performed for continuous variables. Two-tailed hypothesis tests were performed; a p-value <0.05 was considered to be statistically significant.

3. RESULTS

the Clinical Characteristics of the Participants In total, six couples with a history of recurrent TFF after ICSI were included during this study, and also the details of the characteristics of the couples square measure presented in Table one. the average age of all the females was 28.8 ± 1.9 years old. of these couples had never pregnant before with the infertility period starting from 2 to 6 years,

and most of them were diagnosed with unexplained infertility though the numbers of the retrieved oocytes, starting from 8 to 20, were at an average level or perhaps thought-about in terms of the female age, only 1 oocyte or less were fertilized with a very low fertilization rate, which was

TABLE 1 | The clinical characteristics of the enrolled cases.

	Age (female/male)	Type of infertility	Infertility Duration (year)	Causes of infertility	IVF/ICSI cycle	No. of oocytes retrieved	No. of MII oocytes	No. of fertilized oocytes
Case 1	27/30	Primary	2	oligo spermia	ICSI	19	15	1
Case 2	27/28	Primary	4	unexplained	ICSI	8	6	0
Case 3	32/32	Primary	8	unexplained	ICSI	16	8	0
Case 4	30/31	Primary	6	unexplained	ICSI	11	10	1
Case 5	30/33	Primary	5	unexplained	ICSI	9	9	0
Case 6	27/35	Primary	8	unexplained	ICSI	15	7	0

TFF, total fertilization failure. Conventional IVF. ICSI-AOA, ICSI with standard assisted oocyte activation using Ca-ionophore A23187.

Considered as unsuccessful or low fertilization after ICSI) thus, of these patients have try a minimum of one-time ICSI, Oligopermia can be determined just in case 1, and Cases 4 and 5 were detected as teratozoospermia (Supplementary Table 1)

The seminal fluid parameters of the other cases were in the normal range in terms of concentration, motility, and morphology.

The Validity Evaluation of the AOA

Method As shown in Table 2, statistically significant differences can be observed among these different groups in terms of the fertilization rate. Compared with the regular ICSI group and ICSI-AOA oocyte activation method, using calcium ionophore, can significantly increase the fertilization rate in most cases.

Data were shown in number of fertilization oocytes/number of MII oocytes(percentage). Conventional ICSI-AOA: Ca²⁺-ionophore A23187*p-values <0.05 were considered to be statistically significant

Application of the AOA treatment was able to ensure a successful fertilization for couples suffering from TFF after ICS

TABLE 2 | The comparison of the fertilization rates using different methods.

	ICSI	ICSI-AOA	p-value
Case 1	1/15 (6.7%)	7/16 (43.8%)	0.571
Case 2	0/6 (0.0%)	4/7 (57.1%)	0.033*
Case 3	0/8 (6.7%)	1/8 (12.5%)	0.747
Case 4	1/10 (10.0%)	8/18 (44.4%)	0.034*
Case 5	0/9 (0.0%)	7/12 (58.3%)	0.610
Case 6	0/7 (0.0%)	1/2 (50.0%)	0.056

4. DISCUSSION

During this study, we presented AOA technique applied in six couples complains a failing fertilization after previous ICSI, explored its potential mechanism. Our data showed assisted oocyte Activation Method (Ca ACTIVATION) will effectively improve the fertilization rates and promote a normal embryonic development for couples who experienced repeated ICSI failure.

ICSI has been the foremost effective and successful and productive technique to treat male factor infertility like obstructive azoospermia (Rubino et al., 2016)

Despite the high success rate of ICSI, complete fertilization failures still occur in 1–3% of the overall ICSI cycles (Flaherty et al., 1998)

Gamete immaturity, abnormal morphology, or hereditary genetic defects could account for the unsuccessful fertilization (Nasr-Esfahani et al., 2010), and failure of oocyte activation could also be the most reason for TFF (Tesarik and composer, 1995)

In recent years, AOA was advocated to deal with fertilization failure after ICSI, and lots of studies have shown that the bulk of couples experiencing ICSI failure have the benefit of AOA (Montag et al., 2012; Sfountouris et al., 2015; Fawzy et al., 2018). Ca-ionophore is still one in all the most common and effective AOA methods worldwide (Swann, 2018).

5. CONCLUSION

In summary, we found that assisted oocyte Activation

Method (Ca ACTIVATION) was able to effectively improve the fertilization rate within the majority of patients affected by TFF. Remarkably, despite that the sure mechanism of TFF remains unclear, our data showed that this AOA had a possible therapeutic impact on those couples experiencing TFF after ICSI, which can overcome the severe fertilization deficiencies in patients with recurrent low fertilization or TFF.

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