Original Article

Effect Of Chemical Activation On Oocytes And The Optimum Time On Icsi Outcome

Sara Ali El-Gezeiry[1], Omaima Ahmed Khamiss[2] and Mohammed Fadel El-Moher[3]

[1]Saraelgezeiry22@gmail.com
[1&2] Genetic Engineering and Biotechnology Research Institute, Sadat City University.
[3] IVF Lab Director of Dar El Om Center, Cairo.
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Abstract

The problem of infertility is one of the most difficult problems facing humanity. Out of every six families, one family suffers from non-pregnancy. This means that it is a problem that must be speeded up to find out its causes in order to know its treatment.

The purpose of this study, is to discover the optimal way to activate the eggs chemically during the Intracytoplasmic Sperm Injection (ICSI) process by placing the eggs after ICSI in a culture dish containing (calcium ionophore) for ten minutes or injecting the sperm into the ova or egg and then placing the egg that was injected into the calcium ionophore for ten minutes With the work of another group, it is injected in the traditional way without using any stimulants (calcium ionophores), and then placing the three groups in the culture dish and separating them in the nursery to follow up on the fertilization, divisions, and degrees of the embryos.

Keywords: ICSI- Calcium Ionophore - Oocyte Activiation- Male Factors

Introduction

Oocyte activation is a fundamental step for the success of animal cloning. Activation consists of artificially stimulating the oocytes to initiate embryonic development.

Recently, calcium ionophore has been widely used in various centers and obtained satisfactory results. Yet, considering that calcium oscillation is consistent with the active and rapid demethylation of the paternal genome and passive DNA demethylation of the maternal genome, it appears reasonable that an altered calcium signal, as reported following calcium ionophore treatment, might be related to modifications in genomic imprinting **(Nikiforaki et al., 2016)**. Moreover, several meta-analysis studies assessed the value of calcium ionophore during Artificial Oocyte Activation (AOA) in improving reproductive outcomes, and researchers found that ICSI - Artificial Oocyte Activation (ICSI-AOA) played a positive role in enhancing fertilization rate and clinical pregnancy outcome (**Sfontouris et al., 2015; Murugesu et al., 2017**). However, the small number of research samples reduces the credibility of (AOA) effectiveness. Identifying and evaluating more cases would contribute to more reliable conclusions.

The effect of ICSI - Artificial Oocyte Activation (ICSI-AOA) on post-implantation embryo development and pregnancy effectiveness must be taken into consideration deeply. With the widespread application of chemical activators, their safety in offspring has attracted more and more attention. In this meta-analysis, we aim to identify, appraise, and assess the efficacy and safety of calcium ionophore as a method of Artificial Oocyte Activation in promoting pregnancy outcomes and reducing miscarriage and congenital birth defects.

ICSI has become the most popular assisted reproductive technique applied for several infertility indications (**Bonte et al., 2019**). Although normal fertilization rates are estimated at 70%, total fertilization failure (TFF) still occurs in 3%–5% of all Intracytoplasmic sperm injection (ICSI) cycles (**Bhattacharya et al., 2013**). The main cause for Total Fertlization Failure (TFF) after Intracytoplasmic sperm injection (ICSI) has been attributed to oocyte activation deficiencies (OADs), which can be related to sperm or oocyte factors (**Kashir et al., 2010 and Meerschaut et al., 2014**).

Oocyte activation is a universal process comprising a complex series of molecular events that are essential for fertilization (**Stricker, 1999**). The initiation of the oocyte activation process is triggered by a sperm oocyte-activating factor, Phospholipase C Zeta (Plcz), which is delivered into the oocyte at sperm-egg fusion (**Saunders et al., 2002**).

Calcium ionophore treatment during assisted oocyte activation (AOA) is a common chemical activation method and is always used in cases with low fertilization rates or developmental block. Several studies showed that the use of Assisted Oocyte Activation (AOA) with calcium ionophore treatment improved pregnancy and live birth rate to a certain degree. As the number of studies increase, it is necessary to find more reliable evidence of calcium ionophore in improving pregnancy outcome. In this study, a meta-analysis was performed to explore the efficacy and safety of calcium ionophore treatment during Assisted Oocyte Activation (AOA) in improving pregnancy outcome and decreasing the incidence of congenital birth defects (**Shan et al., 2022**).

The present study aimed to evaluate the effect of human artificial oocyte activation by calcium ionophore in improvement of Intracytoplasmic Sperm Injection (ICSI) outcomes.

Material & Methods

Confidentiality:

The confidentiality of all patients admitted to the study was protected. The study participants will not be identified by name in any report or publication resulting from data collected in the study.

Patient selection:

A total of 50 cycles of 50 couples enrolled in the ICSI program in Dar Al- Om Fertility Centre were admitted to this study. cases of severe Oligo-astheno-teratozoospermia or nonobstructive azoospermia or totally immotile spermatozoa or patients who previously had total failure or limited fertilization after ICSI were selected for this study.

Consent:

Written consent was taken from all the patients and the study was performed according to the norms of the Institutional Ethical Committee. All study participants were blinded to treatment assignment for the duration of the study.

The following groups were formed: Design: Oocytes were divided into three groups

- **Group I** (Control group).

Inject 5 oocytes and wash them then transfer to culture dish.

- **Group II**

Put 5 oocytes in amount of calcium ionophore before ICSI and then inject, wash them then transfer to culture dish.

- **Group III**

Inject 5 oocytes and put them in drop 10 micron of calcium ionophore to 10 minutes then wash and transfer to culture dish.

Stimulation protocols:

All patients should receive luteal support with progesterone starting on the day following oocyte retrieval **(Zegers Hochschild et al., 2009).**

Semen Preparation:

Semen analysis was performed in compliance with the World Health Organization 2021**(WHO 2021)** guidelines, which included assessment of the number of sperm cells per milliliter, sperm concentration, sperm motility and finally sperm morphology. The sample is centrifuged at 1300 g for 15 minutes The final pellet was re- suspended in 0.2 ml of (hams f10 supplemented with 10% HAS The pellet is then re-suspended in fresh culture medium, then the semen sample were immediately taken for ICSI procedures.

Oocyte Retrieval:

The patient reports to the assisted conception unit on the morning of the procedure. She should be fasting for at least five hours; ultrasound can is carried out and oocytes are aspirated from follicles in both ovaries through a needle that is used to pierce the vaginal wall and puncture the follicle **(Palermo et al., 1992).**

Intracytoplasmic Sperm Injection (ICSI) procedure:

Oocyte morphology was assessed using an inverted Olympus microscope with a Hoffmann modulation contrast system under 400 × magnifications, just before sperm injection. Mature oocytes (MII) that were observed to have released the first polar body were considered mature and were used for ICSI **(Ekart et al., 2013).**

Fertilization, cleavage and Embryo's quality:

On Day one after injection, zygotes assessed at around 16 hours post injection under Nomarski differential interference contrast optics. Pronuclei (PN) phase was also examined; abnormal zygotes that have monopronuclear (1PN) or tripronuclear (3PN). Evaluate blastocyst-stage morphology Early Blastocyst, Blastocyst or Hatching Blastocyst Stage Blastocysts detected on day five and day six post insemination. It doesn't mean that all were seen on these days scored as blastocysts. There is morula, morula containing cavitations, and compacting cells that couldn't convert into morula stage **(Richardson et al., 2015 and Fadel et al., 2014).**

Statistical Analysis and Sample Size: Student's t-test the was used to compare continuous variables, whereas a chi-squared test was applied to discrete variables. $P < 0.05$ was considered statistically significant.

Results

Effect of activation on ICSI outcomes.

In the terms of ICSI outcomes after activation, all parameters had significant differences between all groups. Most importantly, the number of GRADE A and B embryos were higher with activation, either before or after activation, while the number of Grade C embryos was higher in not activated group. The number of Grade C embryos in the group got activation after injection was significantly lower than those got it before injection ($P<0.001$). Furthermore, the number of Grade A embryos was significantly higher in Group (A=After activation) than in Groups (BA=Before Activation) and (NA= No Activation) ($P<0.05$). See table 1 & Fig 1.

Table (1): Effect of activation on ICSI outcomes.

Variable	Frequency (%)	Not activated (NA)	Activated before injection (BA)	Activated After injection (A)	P-value#		
					P1	P2	P3
Injected oocytes	48 (100%)	5.96± 2.04	6.04± 2.00	6.02± 2.04	<0.001	<0.001	<0.001
Fertilized oocytes	48 (100%)	3.85± 1.75	3.71± 1.97	3.79± 2.01	0.001	<0.001	<0.001
Cleaved oocytes	41 (85.4%)	3.68± 1.91	3.63± 2.09	3.63± 2.12	0.003	<0.001	0.001
Grade A embryos	41 (85.4%)	1.17± 1.28	1.07± 1.10	1.22± 1.26	0.011	0.002	0.034
Grade B embryos	41 (85.4%)	1.83± 1.28	1.9± 1.39	1.90± 1.20	<0.001	0.001	0.014
Grade C embryos	41 (85.4%)	0.66± 1.32	0.63± 1.41	0.51± 1.31	<0.001	<0.001	<0.001

One-way ANOVA, P1: NA versus BA, P2: NA versus A, P3: BA versus A. P<0.05 was considered significant.

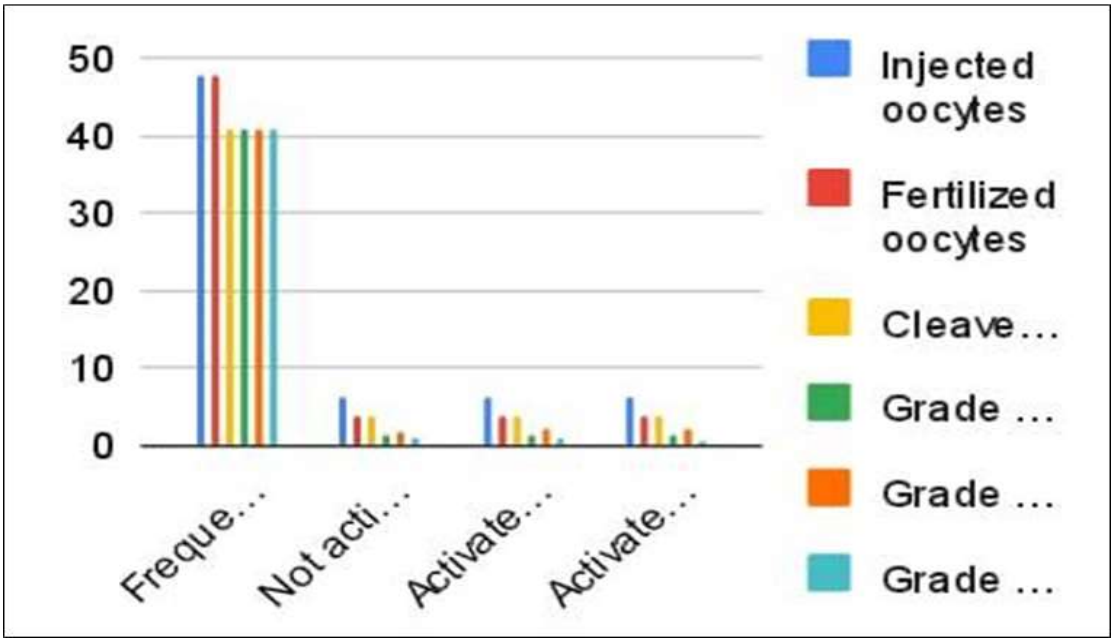

Figure (1): Analysis of Effect of activation on ICSI outcomes.

DISCUSSION

Intracytoplasmic Sperm Injection (ICSI) is one of the new techniques that contributed to the treatment of many cases of infertility due to lack of number, abnormalities or lack of sperm motility, but in some cases, we still face total fertilization failure or fertilization of very few eggs to be microscopically injected and recent research explains the failure of fertilization by the inability of sperm to activate the eggs. Therefore, scientists resorted to the use of techniques to activate eggs during the ICSI process after injection, including hydraulic activation **(Haddad et al., 2021).**

Oligo-asthenozoospermia (OA), also called oligo-asthenospermia, is a primary cause of male infertility where two seminal parameters are altered: sperm count and sperm motility. Thus, oligo-asthenozoospermia is the resulting disorder when oligo-zoospermia and astheno-zoospermia are combined, which aggravates infertility in the male affected. Achieving natural pregnancy with oligo-asthenozoospermia is complicated, but the good news is that it is

possible. Moreover, when conceiving is not possible, these couples can turn to reproductive technologies like In Vitro Fertilization (IVF) to become parents (**Zhu et al., 2021**).

In the present results revealed that, in the terms of ICSI outcomes after activation, all parameters had significant differences between all groups. Most importantly, the number of GRADE A and B embryos were higher with activation, either before or after activation, while the number of Grade C embryos was higher in not activated group. The number of Grade C embryos in the group got activation after injection was significantly lower than those got it before injection.

The explanation for this is that normal sperm fertilization can induce multiple calcium oscillations (**Marangos et al., 2003 and Niu et al., 2020**), while calcium ionophore can induce only one calcium oscillation (BR22) and one calcium oscillation may be not enough to initialize oocyte activation events in some oocytes. Furthermore, oocyte activation is also affected by other factors, such as oocyte maturation status (**Enjoji et al., 2015**, **Economou et al., 2017 and Niu et al., 2020).**

The present study aimed to evaluate the effect of human artificial oocyte activation by calcium ionophore in improvement of ICSI outcomes. In order to improve clinical outcomes for patients with male factor infertility, embryo developmental block, and those who stay infertile after ICSI, assisted oocyte activation (AOA) in conjunction with ICSI has been created and widely used. **(Kang et al., 2015; Ferrer-Buitrago et al., 2018)**. Assisted Oocyte Activation (AOA) techniques include electrical, mechanical, and chemical activation; the most popular and effective of these is chemical Assisted Oocyte Activation (AOA) , which recruits calcium ions from outside the oocyte as well as increasing intracellular Ca2+ to activate oocytes. **(Vanden Meerschaut et al., 2014; Yeste et al., 2016)**

Moreover, several meta-analysis studies assessed the value of calcium ionophore during Assisted Oocyte Activation (AOA) in improving reproductive outcomes, and researchers found that Intracytoplasmic Sperm Injection-Assisted Oocyte Activation (ICSI-AOA) played a positive role in enhancing fertilization rate and clinical pregnancy outcome **(Sfontouris et al., 2015; Murugesu et al., 2017).** However, the small number of research samples reduces the credibility of Assisted Oocyte Activation (AOA) effectiveness.

From the present obtained results, tended to support the notion that calcium ionophore treatment was safe and effective. The analysis results indicated that the injection by calcium ionophore after oocyte activation resulted in a statistically significant improvement in oocyte activation and also promoting pregnancy outcomes and reducing miscarriage and these results are agreed with **Shan et al., 2022)**

CONCLUSION

These previous studies demonstrated that Assisted Ooctyte Activation (AOA) with calcium ionophore after ICSI had no effect on the frequency of miscarriages and congenital birth defects. There is compelling proof that using calcium ionophores to enhance oocyte activation is effective and safe. According to this meta-analysis, partners with low ICSI fertilization rates may benefit from using calcium ionophore to activate oocytes. Thus, in the event that ICSI-induced infertility results in failed fertilization, we advise regular use of Assisted Oocyte Activation (AOA) with calcium ionophore.

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