



Ca Ionophore Vs. Standard Culture Media In Cryopreserved Semen Of Iraqi Patients Undergoing ICSI Cycles

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ABSTRACT

Background: ICSI is increasingly used instead of traditional IVF for all IVF-using couples for several reasons. It has already successfully activated mammalian oocytes with an artificial oocyte activator called calcium ionophores. There is a growing trend to use such activators to address the many covert causes of ineffective fertilization and improve embryo quality. The fear that fertilization might not happen is one of the most important ones.

Aim of the study: to analyze the effects of artificial oocyte activation on the outcomes of ICSI procedure when cryopreserved semen is used.

Material and methods: A total of 17 patients who were selected and subjected to intra-cytoplasmic sperm injection procedure, after controlled ovarian stimulation and ovum pick oocyte immediately following Intracytoplasmic sperm injection were divided into two groups (oocyte with ca ionophore and without ca ionophore) then fertilization rate and embryo quality were checked to each group.

Result: The study involved 162 oocytes, 83 in the ca ionophore group and 79 in the control group. The fertilization rate did not show any noteworthy difference between the two groups. Moreover, there was no significant statistical difference between the two groups regarding embryo grades 1, 2, and 3.

Conclusion: Artificial oocyte activation did not benefit fertilization rate nor embryo quality when cryopreserved semen was used for insemination.

Keywords: *Artificial oocyte activation, in vitro fertilization, intracytoplasmic sperm injection*

INTRODUCTION

Human in vivo or conventional in vitro fertilization (IVF) involves the penetration of cumulus cells, binding and penetration of sperm and oocyte, a fusion of sperm and oocyte, activation of the oocyte, processing of the sperm, and pronucleus production. The pre-sperm-penetration periods' Events 1 through 3 can be prevented with intracytoplasmic sperm injection (ICSI). Thus, ICSI patients experience fewer attempts at fertilization than IVF patients. (1)

The average fertilization rate is between 60 and 70 per cent, even though ICSI passes key crucial stages of typical fertilization. The failure of ICSI to result in pregnancy may be attributed to errors in the oocyte, sperm, and ICSI process. Even if there was sperm inside and over 80% of oocytes after ICSI failed to fertilize, this is probably because the oocyte was not activated or could not decondense the sperm. (2)

In the past, it was commonly thought that basic sperm parameters or paternal factors did not influence the effectiveness of ICSI. However, recent studies have indicated that repeated failures following ICSI may be due to the effects of sperm-related factors on the development of preimplantation embryos. (3)

The natural physiology of sperm can be negatively impacted by cryopreservation, leading to changes and damage that finally result in sperm mortality and lowering freeze-thawed quality criteria. (4)

One of the earliest stages of fertilization is known as oocyte activation, which is characterized by “two fundamental molecular events: an increase in intracellular calcium concentration due to the endoplasmic reticulum's initial release (which happens 1-3 minutes after the sperm binds to the oolemma) and a transient calcium elevation in the oocyte as a result of the steps taken for meiotic resumption during fertilization”. (5)

Several researchers have experimented with various chemical, electrical, and mechanical strategies for artificially stimulating oocytes to overcome fertilization failure following ICSI. The furthestmost widely used technique for artificial oocyte activation is chemical activation. Several chemicals have been demonstrated to

raise intracellular calcium levels and activate oocytes, including “ethanol, calcium ionophore (A23187), ionomycin, puromycin, strontium chloride, probol ester, and thimerosal”. Several studies have shown that calcium ionophores enhanced intracellular free calcium during artificial oocyte activation, simulating the physiological mechanisms that drive oocyte activation. (6, 7)

MATERIALS AND METHODS

Between November 2020 and September 2022, this prospective randomized controlled lab experiment was carried out at the “High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrian University”. The local medical ethical committee approved the trial, and patients gave their written consent to participate.

The study involved 17 patients who underwent an intra-cytoplasmic sperm injection (ICSI) procedure. Before the procedure, each patient underwent a history taking and a complete physical examination. The antagonist protocol was used for all patients, starting with the administration of follicle-stimulating hormone (FSH) on day 2 of the menstrual cycle, followed by the administration of a GnRH antagonist (Cetrorelix acetate for injection 0.25 mg: Cetrotide®, Merk, Switzerland) using the flexible method.

The doses of gonadotrophins were individualized according to serum oestradiol (E2) measurement and transvaginal ultrasound monitoring of the developing follicles. Serial transvaginal ultrasounds were performed to monitor the size and number of follicles, with serum E2 levels measured until the Human chorionic gonadotropin (hCG) trigger. Ovulation was triggered when at least three more 17mm follicles were found, and E2 levels indicated a satisfactory follicular response. Oocytes were retrieved using an aseptic technique and denuded. Sperms were frozen using the sperm freeze Fertipro and Belgium procedure, with the classification of oocytes according to their maturity level.

A solution of A23187 was prepared and placed in a dish with a standard culture medium to activate

the oocytes. The dish was then covered with mineral oil and placed in a CO₂ environment for 2-4 hours before use. The A23187 solution was commercially available from Gynemed (Lensahn, Germany) and prepared in a droplet size of 30- μ l.

The study group was randomly divided into two groups: the artificial oocyte activation (AOA) group, which was carried out using Ca-ionophore, and the control group. The oocytes injected in the AOA group for each patient were exposed to a pre-prepared bicarbonate-buffered reagent that included CaI (GM508 Cult-Active, Gynemed, Lensahn, Germany) for 15 minutes after ICSI based on the manual instructions. Following at least two rinses, the oocytes were transferred to new microdroplets of fertilization medium (6701 SAGE 1-step™) that contained 5% (w/v) human serum albumin (HSA).

All embryos were cultivated for 1-2 days at 37°C in an atmosphere with a humid environment, 6% CO₂, 5% O₂, and 5% O₂. Two visible pronuclei and two polar bodies were regarded to indicate proper fertilization 16–18 hours after ICSI. Fertilization rates were calculated as “(number of

fertilized oocytes/numbers of metaphase II oocytes injected) \times 100”. Embryos were transferred based on best embryo quality irrespective of the group, and pregnancy was confirmed by measurement of β -hCG. Women were supported with progesterone transvaginally until the pregnancy test measurement 14 days after embryo transfer. Data analysis was performed using Statistical Package for Social Sciences (SPSS) version 25, and the results were presented as mean, standard deviation, and ranges.

RESULT

This study involved 17 couples who had experienced infertility and were undergoing ICSI treatment using the antagonist protocol. A total of 162 mature oocytes were analyzed, with 83 belonging to the group treated with ca ionophore and 79 to the control group.

Table 1 shows that the fertilization rate did not differ significantly between the Ca ionophore and the control groups (p-value = 0.828).

TABLE 1: Comparison of fertilization rate between calcium ionophore and control groups when cryo sperm was used.

	Fertilization Rate		P Value
	Ca Ionophore Group Mean \pm SD	Control Group Mean \pm SD	
Frozen Sperm	68.86 \pm 21.97	67.60 \pm 27.89	0.828

Table 2 shows no statistically significant difference in grade 1 between the Ca Ionophore and the control groups, with a p-value of 0.525.

TABLE 2: Comparison of grade 1 embryos between the ca ionophore and control groups.

	Ca Ionophore group	Control group	Total	P value
Number of grade 1 embryos	36	44	80	
Mean \pm SD	1.6364 \pm 1.6775	2 \pm 2.0702		0.525

Table 3 indicates no significant difference in the quantity of grade 2 embryos between the groups administered with Ca ionophore and the control group.

TABLE 3: Comparison of grade 2 embryos between the ca ionophore and control groups.

	Ca Ionophore group	Control group	Total	P value
Number of grade 2 embryos	26	15	41	
Mean \pm SD	1.1818 \pm 1.6224	0.6818 \pm 1.2492		0.258

Table 4 shows no statistically significant difference in grade 3 embryos between the Ca ionophore and control groups.

TABLE 4: Compares grade 3 embryos between the ca ionophore and the control groups.

	Ca Ionophore group	Control group	Total	p-value
Number of grade 3 embryos	11	12	23	
Mean \pm SD	0.5 \pm 1.0579	0.5455 \pm 1.4385		0.905

DISCUSSION

Only a few research looked into how AOA affected frozen sperm. In the current study, there was no significant difference in fertilization rates across the groups, and utilizing AOA had no positive effect on embryo quality. This is because cryopreservation hurts sperm quality. The most frequently observed result is decreased sperm motility; the exact mechanism causing this harm is still unknown. However, it may have a physical-chemical origin. It might be connected to both osmotic damages brought on by significant cell shrinkage and osmotic stress and intracellular ice crystal formation during cryopreservation. Also, it was hypothesized that cryopreserving sperm causes serious harm to their morphology, chromatin, and membrane integrity. (8)

This non-significant difference is also explained by the above-mentioned deleterious impact of cryopreservation on aspects of the fertilization process other than the activation process, including DNA fragmentation and chromatin damage. These hazards cannot be improved by AOA usage, as it affects the genetic material of these sperms.

Returning to the literature review, we can observe that the Borges et al. study examined the potential role of AOA in cryopreserved sperms from patients who had undergone surgical sperm retrieval. It was shown that neither obstructive

testicular sperm extraction (TESE) nor non-obstructive TESE patients demonstrated higher fertilisation rates, implantation, and pregnancy rates when A23187 was employed. (3)

However, in another study, Borges showed that the limited benefit of using Ca ionophore in patients with cryopreserved azoospermic samples is probably in younger patients (<36 years of age), those with ejaculated and epididymal spermatozoa by achieving better embryo quality. (9)

It may be possible to explain why oocytes injected with ejaculated and epididymal spermatozoa should respond favourably to AOA if there is a link between sperm age and oocyte activation function. Those who had testicular sperm injections, however, were unable to. One of these theories holds that PLC-Z is either present in lower amounts or is inactive in immature spermatozoa as opposed to mature or partially mature spermatozoa. As a result, it is also conceivable to predict that after artificial stimulation of oocyte activation, a testicular spermatozoon cannot maintain the calcium oscillations in the ooplasm. (10)

On the contrary, Ebner et al. study showed that cryopreserved azoospermic male patients benefited tremendously ($P < 0.001$) concerning fertilization, implantation, clinical pregnancy, and live birth rates. (11)

Similarly, the researchers Karabulut et al. suggested the possible advantages of using AOA to stimulate the development potential of fertilized eggs by frozen sperm. They conducted a study that included four patients who underwent ICSI with AOA: those with a history of previous testicular sperm extraction, low oocyte count, severe sperm quality, and frozen sperm (FS) group. Although the rates of fertilization and embryo quality were higher in the AOA group than in the control group, the differences were not statistically significant. However, the rates of top-quality embryos increased by 27.7%, but the differences were still insignificant. (12)

Despite contrasting results, these results provide insight into the role of artificial activation in cryopreserved samples, guiding us to more investigations to reach the answer by conducting more extensive trials or meta-analyses.

CONCLUSION

Artificial oocyte activation did not benefit fertilization rate or embryo quality when cryopreserved semen was used for insemination.

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