African J. Biol. Sci., 20 (2): 67-78(2024)
ISSN 1687-4870 **e- ISSN 2314-5501 (online)**
www.ajbs.journals.ekb.eg **E-mail:**aasdjournal@yahoo.com

Activation of human oocytes after previously intracytoplasmic sperm injection (ICSI) fertilization failure

Osama M. Badr[1*], Mohamed Shehata[2], Eman Anwar Hassan[2] and Hoda Gamal Alden[1]
[1] Animal Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City (USC)
[2]International Islamic Center for Population Studies and Research, Al-Azhar University.
*Corresponding author E-mail: osama.badr@gebri.usc.edu.eg

Received: August 2, 2024; Accepted: August 25, 2024; Available Online: August 25, 2024

ABSTRACT

Infertility is a major problem that affects the individual and society. The current investigation aimed to investigate the efficiency of activation with ionomycin or 7% ethanol methods to assist oocyte for improving intracytoplasmic sperm injection (ICSI) outcomes in cases of recurrent failure of ICSI including severe male factor infertility, previous failure of fertilization and implantation. Both methods of assisted oocyte activation (AOA) used in this study recorded highly efficient implications on infertility duration, mature and fertilized oocytes, rates of fertilized, cleavage, blastocyst, embryo grades and pregnancy. It was found that using Ionomycin was preferable more than 7% Ethanol in patients' treatment with failure fertilization with ICSI.

Keywords: ICSI, AOA, Infertility, Fertilization.

INTRODUCTION

The assisted reproductive technology is infertility therapy where sperm and oocytes are manipulated outside the body, like *in vitro* fertilization and ICSI. The latter is a micromanipulation technique employed to inject sperm directly into the oocyst cytoplasm. ICSI is preferred procedures to address male infertility when other methods of assisted fertilization have been unsuccessful. It has become possible by applying ICSI to fertilize oocytes from patient whose partner possess very low amount of viable sperms and virtually there is no chance to achieve fertilization using traditional in vitro spermatocyte incubation methods (Graham *et al.,* 2023).

Failed fertilization generally refers to the failure of all accessible mature metaphase II oocytes to undergo fertilization. Disruption in oocyte activation, whether due to dysfunction in the male or female gamete, was considered the primary cause of failed fertilization. The occurrence of total fertilization failure following ICSI ranges from 1-5% (Zhang *et al.,* 2023b).

Fertilization failure after ICSI can attributed to: a) failure of the injected oocyte to initiate the biochemical processes required for its activation, b) the biochemical processes may not proceed normally, c) sperm is inadequately accessible to the oocyte factors necessary for condensation of chromatin and forming male pronuclei. It is thought that unsuccessful oocyte activation following ICSI is caused by both oocyte and sperm factors (Tang *et al.,* 2022).

In human-assisted reproduction treatments, different artificial activation methods, such as mechanical, physical, or chemical stimuli were employed to induce rising in calcium in the cytoplasm of the oocyte. Artificial activating agents like ionomycin or calcimycin (A23187) (calcium ionophores) represent one of the most employed methods to assist human reproduction (Sun and Yeh, 2021).

Ionomycin is produced by microorganisms and it transfers ions through the cell membrane lipid bilayer (Zaratsky and Zaretskaia, 2020). The calcium ionosphere promotes oocyte activation by enhancing the cell membrane's calcium permeability, which allows extracellular calcium to enter the cell. Furthermore, from intracellular stores calcium is released.

MATERIALS AND METHODS

The study involved a cohort of 200 couples who had been directed to the assisted reproduction unit at the Fertility Clinic, Al Azhar University, during the period from September 2018 to September 2020. All subjects who undergo ICSI cycle reveal no previous indication of successful fertilization (absence of 2 pronuclear formations and extrusion of the second polar body) after 24 hours following ICSI.

Ethical approval:

The written informed consent and the protocol and were approved from the ethics committee at the International Islamic Centre for Population Studies and Research (Al-Azhar University, Egypt). All participants gave informed written consent. This study was conducted under the Registration number 39, Faculty of Medicine, Al Azhar University.

Confidentiality:

Every patient who was admitted for the study had his privacy respected. The names of study participants will not appear in any report or publication derived from the project's data collection.

Subjects of the study were classified into two groups:

G1: Females partners (100 cases) undergo ICSI, treated with ethanol (7%) for 6 minutes.

G2: Females partners (100 cases)) undergo ICSI, treated with 5 mmol/l of ionomycin (Sigma) for 5 minutes.

I-Technical approach of all male subjects:

A) Samples collection of semen:

Semen samples were obtained through masturbation following an abstinence period of 2-5 days. Extended abstinence (10 days) tends to reduce motility, while shorter durations lead to lower density and volume. The collection container was sterile, clean and has a wide opening to reduce errors. It was sourced from a batch proven to be non-toxic to sperm. The specimen of semen was maintained body temperature and was examined through time interval of one hour (WHO, 2010).

B)- Physical examination:

- **Appearance of the ejaculate:** Appearance was estimated by visual assessment. A standard semen sample exhibits a uniform, grayish opalescent hue.
- **Liquefaction:** The specimen container was placed at 37°C in an incubator for semen to be liquefied for 15 minutes, or at room temperature for 60 minutes. When liquefaction is not complete during 60-minute timeframe, this observation should be noted (WHO, 2010; Hsu *et al.,* 2023).
- **Semen viscosity:** This was estimated by plastic disposable pipette by gently aspirating (Saravanabavan and Rangan, 2023).
- Semen volume: It was measured by aspirating the whole ejaculate into a sterile, graduated warmed glass pipette (Swidan *et al.,* 2020).
- **Odor:** The scent of semen can be influenced by certain foods or medications excreted through it. In some instances, it may become unpleasant due to genital infections, or it may have a urinous odor if contaminated with urine (WHO, 2010).
- **Semen pH:** It was estimated by pH paper with range from 6.0 to 10.0. The following steps were followed after liquefaction, ideally 30 minutes but no later than 1-hour post-ejaculation:
  - Thoroughly mix the semen specimen.
  - Place a semen drop onto the pH paper.

- Let the colour develop uniformly (within 30 seconds).
- Match the colour to the calibration strip to determine the pH.

C- Microscopic examination:

1-Sperm concentration:

10-µl of semen was transferred using a pipette onto a clean glass slide, then covered with cover slip. Initial microscopic evaluation was done at 100 x as it provides evaluation of mucus strand, sperm clustering and spread of spermatozoa on slide, followed by a more magnified picture at 400x. One sperm in microscopic field at 400X equals roughly to 1 million/ ml of seminal fluid **(Preisinger *et al.,* 2023).**

2-Sperm motility:

Well mixed undiluted semen drop was put on the surface of warm, dry, and clean microscopic slide and then cover slip is placed. The slide then allows to rest on the bench or on the microscopic stage until stoppage of fluid movement. The drop of semen was examined at magnification of 400x using a microscope equipped with phase contrast. Spermatozoa, including both motile and immotile types were evaluated in at least five distinct microscopic fields, at minimum 200 spermatozoa should be assessed. The percentage motility was calculated from the mean value (WHO, 2010).

3- Categories of sperm movement:

THE Type of sperm motility was determined according to WHO (2010) as follow:

- Progressive motility (PR): Spermatozoa exhibits active movement.
- Non-progressive motility (NP): Spermatozoa display movement patterns without forward progression.
- Immobility (IM): No movement

4- Sperm morphology:

Evaluating sperm morphology with rigorous standards involves a thorough process, beginning with preparing clean microscope slides, properly making thin semen smears, and assessing the slides (Liu *et al.,* 2023).

II- Sperm preparation technique:

All males were subjected to prepare semen by Swim-up technique as follow: Semen should ideally remain undiluted and uncentrifuged before swim-up, as dilution and centrifugation can cause oxidative damage to sperm membranes. Therefore, directly performing a swim-up to isolate motile spermatozoa is the recommended approach (WHO 2010).

Procedure:

1. Mixed the semen specimen.
2. Transferred 1 ml of semen into a sterile conical centrifuge tube (15-ml) and carefully overlay 1.2 ml of the supplemented medium.
3. The tube was tilted at a 45°angle to expand the Semen–culture medium interface and was incubate at 37 °C for an hour.
4. The tube was repositioned upright and the topmost 1 ml of medium was eliminated. This will comprise increasingly mobile sperm cells.
5. The sample is ready for immediate therapeutic or research applications.

III- Technical approach of female subjects:

Oocyte collection, identification, grading, and denudation

1- Stimulation protocols:

The cases treated with suitable superovulation program to get an enough number of eggs.

a-Agonist protocol:

In the long mid-luteal protocol using a GnRH agonist (agonist group), a daily injection of 0.1 mg triptorelin (Decapeptyl, Ferring, Egypt) was initiated on day 21 of the preceding cycle, which was 3 days before discontinuing oral contraceptive pills for 2 weeks. Complete down-regulation was confirmed if the estimated E2 level was below 50 pg/ml. Subsequently, rFSH, recombinant FSH, in the form of Gonal-F (Follitropin b, Merck Serono, Egypt) was administered at an initial dose (150 IU/day), with adjustments made based on individual response. The

administration of both the agonist and recombinant FSH was maintained until the triggering day (Zhang *et al.,* 2023a).

b-Antagonist protocol:

In the fixed protocol using a GnRH antagonist (antagonist group), rFSH was initiated on cycle day 2 at a daily dose of 150 IU (adjusting based on response). On day 7, the antagonist (Cetrotide, cetrorelix 0.25 mg, Merck Serono, Egypt) was introduced, and both rFSH and the antagonist were maintained until trigger day (Wang *et al.,* 2023). Once three follicles attained an average diameter of at least 17 mm, a single i.m. dose of 5000 mIU/mL of Human chorionic gonadotropin (HCG) (Pregnyl, Organon, Netherlands) was given 36 hours prior to ovum retrieval (Yu *et al.,* 2023).

2- Oocyte retrieval:

Oocyte was collected using a single-lumen needle guided by transvaginal ultrasound. The patient came fasting to the operation room and the method was done under general anaesthesia. A vaginal probe was inserted into the vagina, and the pelvis was examined comprehensively to verify the uterus's location, assess the endometrium's condition, determine the ovaries accessibility and position, and count the follicles to be aspirated. An ultrasound probe fitted with a needle guide was inserted into the vagina and directed towards the posterolateral aspect of the vaginal fornix along the digital needle guide set by the Labotect's aspiration pump (Pargianas*et al.,* 2020).

3- Egg Collection:

The complexes of oocyte cumulus cells (OCC) were separated using the dissecting microscope (Zeiss Stemi 2000-C Stereo Microscope) and rinsed in a global total w/HEPES Buffer (Life Global, Europe), then rinsed and transferred into four well dishes with identical medium (Fischer *et al.,* 2023).

4- Denudation:

Oocyte was immersed in 100µl buffered solution encompassing hyaluronidase at 80 IU/ml (Life Global, Europe) for 30-45 seconds. After this, the oocyte was taken out and transferred to a new100µl global total w/HEPES Buffer (Life Global, Europe). Then the corona cells were carefully removed by aspirating the oocyte within and outside a sterile stripper pipette. Once denudation was finished, the oocyte was rinsed with global total w/HEPES Buffer and half number of denudated oocytes was transferred into 10 µl micro drops of the same buffer in injection dishes of ICSI and the other half was then transferred into PICSI injection dishes, covered with 3 ml sterile mineral oil that has been equilibrated (Brickley *et al.,* 2023).

5- The oocyte grading:

The grading system was used to evaluate the grade of oocyte maturity (Quality) with an inverted (Olympus 1x71) microscope equipped with hot stage, Hoffman optics, and automatic manipulators Narishige. The denuded oocytes underwent incubation at 37ºC in 6% CO_2 environment until the ICSI procedure (Bori *et al.,* 2022).

6- ICSI procedure:

Each incubated mature oocyte received a spermatozoon (morphologically normal and mechanically immobilized) in Polyvinylpyrrolidone (PVP). The sperm cells that were treated with ICSI underwent thorough examination and assessment. The procedure for injection was performed in a sterile dish with the use of a holding pipette and an injection needle. The mature oocyte was placed in a 10µl global total with HEPES Buffer (Life Global, Europe), maintained at 37ºC with 6% CO2 and 90-95% humidity, and covered with mineral oil. Sperm was introduced into another 10µl droplet of the same buffer, and the optimal sperm was selected for injection and mechanically immobilized in a PVP droplet (Zhu *et al.,* 2023).

The cytoplasmic sperm injection procedure, following Van Steirteghem's protocol, was executed using an Ax overt 135 equipped with Hoffman optics and 10, 20 and 40x objectives and 10x eyepieces.

Micromanipulators were used to gently apply negative pressure to secure the oocyte with the holding pipette. The sperm, suspended in PVP within the injection needle, was aligned with the focal plane, positioning a single sperm precisely at the needle's tip (Hafezi *et al.,* 2023).

The subsequent step involved a gradual and steady movement into the cytoplasm of the metaphase 2 (MII) oocyte. The sperm, along with around 1 to 3µl of medium, was introduced into the oocyte. After injection, the oocyte was rinsed and placed in global total media (Life Global, Europe) within a culture dish with warm sterile global oil that has been equilibrated (Life Global, Europe). It was maintained at 37ºC in 6% CO_2 in 90-95% humidity until fertilization (Metwalley *et al.,* 2020).

7- Fertilization and embryo's quality assessment:

This includes assessment of rates of fertilization and cleavage,besides embryo grading and pregnancy rate. Fertilization was evaluated 16–18 hours post-microinjection. Observations were made on the injected oocytes for any indications of damage and pronuclei. Oocyte was deemed fertilized if it contained two pronuclei and had expelled the second polar body. Around 72 hours post-microinjection, enough embryos were transferred into recipient subjects. Embryo were assessed and classified into 4 grades based on their cell count and morphology (Mutia *et al.,* 2023). These include:

Grade (A): blastomeres have uniform size and without fragmentation.

Grade (B): blastomeres have slight variations up to 10% cytoplasmic fragments.

Grade (C): blastomeres have notable variations up to 50% fragments and with large granules.

Grade (D): blastomeres have significant variations in size, with substantial fragmentation, large dark granules.

At day 3 embryos were implanted into recipient subjects following the American Society of Reproduction's protocols. High-quality embryos were preserved through cryopreservation. Serum-HCG levels were assessed 14 days post-transfer as a measure of chemical pregnancy (positive if 20 IU/L). After the absence of menstruation, a transvaginal ultrasound scan was done to confirm the presence of a clinical pregnancy, evidenced by the visibility of an intrauterine gestational sac (Jiang and Bormann, 2023).

IV- Statistical analysis:

The current data were analysed using software package **(SAS) and the p**aired T-test and Mc Nomar's test had been used for comparing between parameters in each group. Also, none paired T test and Fischer exact test had been used to compare between the mean changes in the studied parameters for various groups. The significant level was set at $P<0.05$ (Gomez *et al.,* 2023).

RESULTS

1- Sperm parameters in male patients:

The incidence of sperm count in Ionomycin group was 10.80±5.1, while it was 10.12 ± 4.1 in Ethanol (7%) group which indicated a non-significant distinction ($P>0.05$). Also, there was non-significant differences ($P>0.05$) between the prevalence of sperm motility in the Ionomycin group (20.20±5.0) compared to Ethanol (7%) group (21.31±4.8). Similarly, the incidence of abnormal forms of sperm was (99.40± 1.1) in the Ionomycin group compared to (99.18±1.3) in the Ethanol (7%) group which revealed a non-significant difference ($P>0.05$).

Table (1): Sperm parameters in male patients.

Parameters	Ionomycin (N=100)	Ethanol 7% (N=100)	P value
	Mean ± SD	Mean ± SD	
Sperm count (×10^6/ml)	10.80 ± 5.1	10.12 ± 4.1	P > 0.05
Sperm motility/ml	20.20 ± 5.0	21.31 ± 4.8	P > 0.05
Abnormal forms	99.40 ± 1.1	99.18 ± 1.3	P > 0.05

not-significant at P > 0.05

2- General characters of the female patients:

Table (2) showed non-significant distinction (P>0.05) in the distribution of the mean values of the studied female patients age, body mass index (BMI), and previous failed trials. Their mean age was (32.94±4.0) in the Ionomycin group while it was 33.10±4.0 in the Ethanol (7%) group. Their mean BMI was (28.12±3.5) in the Ionomycin group compared to (28.80±5.5) in the Ethanol 7% group. The incidence of Previous failed trials was 2.68±1.0 in the Ionomycin group and 2.51±1.0 in the Ethanol (7%) group. However, the mean duration of infertility of female patients was 7.13±2.1 in the Ionomycin group and 6.61±1.6 in the Ethanol (7%) group displaying a statistically significant difference (P≤0.05).

Table (2): General characters of the female patients.

Parameters	Ionomycin (N=100)	Ethanol 7% (N=100)	P value
	Mean ± SD	Mean ± SD	
Age/ years	32.94 ± 4.0	33.10 ± 4.0	P>0.05
BMI (Kg/m^2)	28.12 ± 3.5	28.80 ± 5.5	P>0.05
Infertility duration/years	7.13 ± 2.1	6.61 ± 1.6	P≤0.05*
Previous failed trials	2.68 ± 1.0	2.51 ± 1.0	P>0.05

(*) significant P ≤ 0.05, not-significant at P > 0.05

3- ICSI outcomes among groups:

It was obvious from (Table 3) that the occurrence of the mean number of the collected oocytes was 9.34±2.1 in the Ionomycin group compared to 0.21± 3.1 in the Ethanol (7%) group which exhibited a statistically non-significant difference (P>0.05). There was a very highly statistically significant (P≤0.001) between the incidence of the mean number of mature oocytes (8.72±1.1) in the Ionomycin group compared to that in the Ethanol (7%) group (8.00±1.0). Also, the mean rate of fertilized oocytes number 5.23±1.5 in the Ionomycin group compared to 4.01±2.1 in the Ethanol (7%) group showed a statistically very highly significant (P≤0.001).

Table (3): Oocytes factors in ICSI outcomes among females.

Parameters	Ionomycin (N=100)	Ethanol 7% (N=100)	P value
	Mean ± SD	Mean ± SD	
Total collected number /case	9.34 ± 2.1	10.21 ± 3.1	P > 0.05
Mature oocytes/case	8.72 ± 1.1	8.00 ± 1.0	P≤ 0.001***
Fertilized oocytes/case	5.23 ± 1.5	4.01 ± 2.1	P≤ 0.001***

(***) very highly significant P ≤ 0.001 not-significant at P > 0.05

4- Rates of fertilization and the cleavage among groups:

It was clear from data in Table (4) the presence of a very highly significant difference (P≤0.001) regarding the incidence of the mean fertilization rate on day (1) (5.23±1.5) in Ionomycin group and 4.01± 2.1 in Ethanol (7%) group, while the mean cleavage rate on days (2 & 3) was 4.99±1.0 in the first group compared to 3.90±0.1 in the second group. Similarly, the incidence of mean blastocyst formation rate on day (5) was 3.73±1.2 in the first group and 2.70±0.1 in the second group with a very highly significant difference (P ≤ 0.001).

Table (4): The fertilization rate and embryos development among female's patients.

Parameters	Ionomycin (N=100)	Ethanol 7% (N=100)	P value
	Mean ± SD	Mean ± SD	
Fertilized rate (D1)	5.23 ± 1.5	4.01 ± 2.1	P≤ 0.001***
Cleavage rate (D2)	4.99 ± 1.0	3.90 ± 0.1	P≤ 0.001***
Cleavage rate (D3)	4.99 ± 1.0	3.90 ± 0.1	P≤ 0.001***
Blastocyst rate (D5)	3.73 ± 1.2	2.70 ± 0.1	P≤ 0.001***

(***) very highly significant P ≤ 0.001

5- Comparison between embryo grading among different groups:

Data in in Table (5) indicated that the incidence of the mean number of **Grade A** embryos was 3.73±1.2 in Ionomycin group compared to Ethanol (7%) group (2.70±0.1) and this difference exhibited a very highly statistically significant (P≤0.001). However, that of Grade B embryos was 1.03±1.0 in Ionomycin group compared to Ethanol (7%) group (1.00 ± 0.1) and this exhibited a statistically non-significant difference (P>0.05). The incidence of mean number of Grade C embryos was 0.33±1.2 in the first group and 0.90±0.1 in the second group with a very highly statistically significant (P≤0.001).

Table (5): Embryo grading in female patients.

Embryo grading	Ionomycin (N=100)	Ethanol 7% (N=100)	P value
	Mean ± SD	Mean ± SD	
Grade A embryos	3.73 ± 1.2	2.70 ± 0.1	P≤0.001***
Grade B embryos	1.03 ± 1.0	1.00 ± 0.1	P>0.05
Grade C embryos	0.33 ± 1.2	0.90 ± 0.1	P≤0.001***

(***) very highly significant P ≤ 0.001 not-significant at P>0.05

6- Rates of pregnancy among female patients' groups:

It was obvious from Table (6) that the pregnancy rate was 49.0% in the Ionomycin group compared to 39.0% in the Ethanol 7% group, reflecting a very highly statistically significant distinction among the studied groups (P≤0.001).

Table (6): The pregnancy rates among female patients (n=200).

Parameters	Ionomycin (N=100)	Ethanol 7% (N=100)	P value
	%	%	
Pregnancy rate %	49.0	39.0	P ≤ 0.001***

(***) very highly significant P ≤ 0.001

DISCUSSION

This study aimed to evaluate the effectiveness of the artificial activation of unfertilized human oocytes utilizing two different methods (7% ethanol and ionomycin); following Intracytoplasmic sperm injection (ICSI). Kasai *et al.* (2002) reported that failure in fertilization still happens in 2-3% of intracytoplasmic sperm injection (ICSI) cycles, despite significant advancements in the clinical and analytical components of the process. Nasr-Esfahani *et al.* (2008) found that lack of oocyte activation is one factor contributing to unsuccessful fertilization following ICSI and they mentioned that a variety of mechanical, electrical, and chemical techniques are employed in these situations to artificially activate oocytes. Currently, chemical oocyte activation using materials like calcium ionophore is one of these techniques to increase the rate of fertilization in ICSI cycles (Mansour *et al.,* 2009). Ethanol and ionomycin are chemical activators that trigger a single increase in calcium levels (Lee *et al.,* 2015). Ionomycin is a member of the class of natural compounds known as polyether antibiotics, having been discovered from *Streptomyces conglobatus* in 1978. Because of its exceptional affinity for binding calcium, ionomycin has found widespread application in neurochemistry as a means of examining the consequences of elevating intracellular Ca^{2+} concentration (Lautens*et al.,* 2002).

The development of assisted oocyte activation (AOA) procedures occurred after research revealed that inadequate oocyte activation was a primary factor in infertility following ICSI. Through mechanical, electrical, or chemical methods, the strategies try to induce increases in intracellular calcium levels. It is thought that this increase in calcium helps oocytes reach a certain threshold needed for successful activation events (Sun and Yeh, 2021).

The most employed artificial activation agent in human assisted reproduction is ionomycin. Ionomycin activates the oocyte by raising the calcium permeability of the cell membrane, which facilitates the influx of extracellular calcium into the cell. In the present study, it was verified if ICSI-AOA is advantageous for cases of recurrent ICSI failure either previous fertilization failure, severe male factor, or implantation failure, these findings were approved with Ruan *et al.* (2023) who concluded that patients with poor embryo growth and past fertilization failure following ICSI may have better reproductive success when using the AOA in combination with the calcium ionophore ionomycin.

The current results showed a very highly statistically significant distinctions in rates of fertilization and the cleavage between the using Ionomycin and 7% Ethanol on improving embryo quality and fertilization rates in ICSI cycles. The occurrence of rates of blastocyst formation on day (5) was 3.73±1.2 in Ionomycin group compared to 2.70±0.1 in Ethanol (7%) group which was in concomitance with Pathak *et al.* (2017), who concluded that the 7% ethanol and ionomycin induced activation in fertilization rates, cleavage rates and blastocyst rates in a statistically significant manner. Sugaya (2010) demonstrated a favorable pregnancy outcome following the stimulation of calcium ionophore A23187 oocytes in an

infertile couple who had previously failed. All the oocytes were not fertilized, nevertheless. Three oocytes were used in the third ICSI attempt, and they were stimulated for five minutes using a calcium ionophore. As a result, two of the three oocytes were fertilized. The subsequent delivery of a healthy child free of congenital defects marked the end of a successful pregnancy. Thus, it was shown that utilizing calcium ionophore for oocyte activation could be helpful when ICSI failed to fertilize an egg multiple time. Darwish and Magdi (2015) reported that the use of chemical AOA employing the A23187 Ca^{2+} ionophore enhanced the embryonic development in four women who had previously had ICSI trials and had a history of a complete fertilization arrest and failure to transit into the cleavage stage. In one of the four couples, the use of activated oocytes improved fertilization, embryonic development, and clinical pregnancy.

The current results indicated that the differences of the embryo qualities; grades A, B and C recorded very highly statistically significant differences between the two studied groups (Ionomycin and 7% Ethanol) ($P \leq 0.001$); these results were in accordance with the findings of (Esbert *et al*., 2022).

The present results agreed with the findings of Jia *et al.* (2023) who found that AOA with 10 µmol/l ionomycin may be more effective than commercial A23187 in improving the patients at risk of failed or impaired fertilization, especially in cases of sperm-related defects. Also, the present results confirmed that the ionomycin is more effective than 7% ethanol in enhancing the fertilized rate, cleavage rate, blastocyst rate and pregnancy rate in a very highly statistically significant difference and these were agreed with those of Barberán *et al.* (2023).

The use of AOA to artificially induce calcium rises by using Ca^{2+} ionophores (7% ethanol and ionomycin) lead to an effective result in overcoming fertilization failure after ICSI (Abdulsamad *et al.,* 2023). These finding was in concomitance with the results of the current study.

Conclusions:

Assisted oocyte activation methods with ionomycin or 7% ethanol to improve ICSI outcome in cases of the recurrent ICSI failure, including severe male factor infertility, previous fertilization failure and implantation failure, gave highly implications on infertility duration, mature and fertilized oocytes, fertilized rates, cleavage rates, blastocyst rates, embryo grades and pregnancy rates. Ionomycin was preferable to more than 7% Ethanol in the treatment of patients with ICSI fertilization failure.

REFERENCES

Abdulsamad, H.M.; Murtaza, Z.F.; AlMuhairi, H.M.; Bafleh, W.S.; AlMansoori, S.A.; AlQubaisi, S.A. and Kashir, J. (2023). The Therapeutic and diagnostic potential of phospholipase C Zeta, oocyte activation, and calcium in treating human infertility. Pharmaceuticals, 16(3):438-441.

Barberán, A.C.; Bonte, D.; Boel, A.; Thys, V.; Paredis, R.; Machtelinckx, F. and Heindryckx, B. (2023). Assisted oocyte activation does not overcome recurrent embryo developmental problems. Human Reproduction, 38(5): 872-885.

Brickley, A.; O'Leary, R.; Lindsay, K.; Aycock, H. and Barritt, J. (2023). Spindle presence and location between denudation and oocyte freezing can change. Fertil Steril., 120(1): e10-e11.

Darwish, E. and Magdi, Y. (2015). A preliminary report of successful cleavage after calcium ionophore activation at ICSI in cases with previous arrest at the pronuclear

stage. Reproductive BioMedicine Online, 31(6):799–804.

Esbert, M.; Carmody, A. F.; Ballesteros, A.; Seli, E. and Scott, R. T. (2022). Calcium Ionophore A23187 treatment to rescue unfertilized oocytes: a prospective randomized analysis of sibling oocytes. Reprod Biomed Online, 45(5):878-883.

Fischer, G. J.; Hartleb, C.; Holmes, K.; Hansum, C. and Tintle, N. (2023). Cisco Aquaculture Best Practices: Randomized Experiments from Eggs to Juvenile. North American Journal of Aquaculture, 85(1):13-20.

Gomez, C.; Goponenko, A.V. and Soulakova, J.N. (2023). Constructing UpSet plot for survey data with weights using SAS and R software. Communications in Statistics-Simulation and Computation, 52(6): 2320-2326.

Graham, M.E.; Jelin, A.; Hoon Jr, A.H.; Wilms Floet, A.M.; Levey, E. and Graham, E.M. (2023). Assisted reproductive technology: Short and long-term outcomes. Developmental Medicine and Child Neurology, 65(11): 38-49.

Hafezi, M., Chekini, Z., Ghaffari, F., Mashayekhi, M. and Zameni, N. (2023). How could one sperm and one oocyte proceed to molar pregnancy? Prevalence of molar pregnancy during ICSI procedure in over 25,000 fresh embryo transfers: a retrospective cross-sectional study. Int. J. Fertil. Steril., 17(1): 47-51.

Hsu, C.T.; Lee, C.I.; Lin, F.S.; Wang, F.Z.; Chang, H.C.; Wang, T.E. and Agarwal, A. (2023). Live motile sperm sorting device for enhanced sperm-fertilization competency: comparative analysis with density-gradient centrifugation and microfluidic sperm sorting. J. Assisted Reproduction and Genetics, 40(8):1855-1864.

Jia, L.; Chen, P.; Su, W.; He, S.; Guo, Y.; Zheng, L. and Liang, X. (2023). Artificial oocyte activation with ionomycin compared with A23187 among patients at risk of failed or impaired fertilization. Reproductive BioMedicine Online, 46(1): 35-45.

Jiang, V. S. and Bormann, C. L. (2023). Artificial intelligence in the in vitro fertilization laboratory: a review of advancements over the last decade. Fertil Steril., 120(1): 17-23.

Kasai, T.; Ogawa, K.; Mizuno, K.; Nagai, S.; Uchida, Y. and Ohta, S. (2002). Relationship between sperm mitochondrial membrane potential, sperm motility, and fertility potential. Asian J Androl.; 4:97–103.

Lautens, M.; Colucci, J. T.; Hiebert, S.; Smith, N.D. and Bouchain, G. (2002). Total Synthesis of Ionomycin Using Ring-Opening Strategies. Organic Letters, 4(11): 1879-1882.

Lee, J. W.; Chang, H. C.; Wu, H. Y.; Liu, S. S.; Wang, C. H.; Chu, C. Y. and Shen, P. S. (2015). Effects of sperm pretreatment and embryo activation methods on the development of bovine embryos produced by intracytoplasmic sperm injection. Reprod. Biol.; 159:1-9.

Liu, K.; Mao, X.; Pan, F.; Chen, Y. and An, R. (2023). Correlation analysis of sperm DNA fragmentation index with semen parameters and the effect of sperm DFI on outcomes of ART. Scientific Reports, 13(1): 1-10.

Mansour, R.; Fahmy, I.; Tawab, N.A.; Kamal, A.; El-Demery, Y. and Aboulghar, M. (2009). Electrical activation of oocytes after intracytoplasmic sperm injection: a controlled randomized study. Fertil Steril., 91:133–139.

Metwalley, A.; Brasha, N.; Esteves, S. C.; Fawzy, M.; Brasha, H.; Hellani, A. and Khamiss, O. (2020). Role of diagnostic intracytoplasmic sperm injection (ICSI) in the management of genetically determined zona pellucida-free oocytes during in vitro fertilization: a case report. Zygote, 28(6): 519-523.

Mutia, K.; Wiweko, B.; Abinawanto, A.; Dwiranti, A. and Bowolaksono, A. (2023). microRNAs as a biomarker to predict embryo quality assessment in *in vitro* fertilization. Int. J. Fertil. Steril., 17(2): 85-91.

Nasr-Esfahani, M. H.; Razavi, S.; Javdan, Z. and Tavalaee, M. (2008). Artificial oocyte activation in severe teratozoospermia undergoing intracytoplasmic sperm injection. Fertil Steril., 90:2231–2237.

Pargianas, M.; Salta, S.; Fiorentzis, S.; Kalampoki, L. G.; Beck, R.; Vizziello, D. and Kosmas, I. (2020). Complications in oocyte retrieval. Pick up and oocyte management, 29(32): 305-337.

Pathak, J.; Kharche, S.D. Goel, A. (2017). Effects of different activation protocols on cleavage rate and blastocyst production of caprine oocytes. Iran J Vet Res.; 18(4): 243–248.

Preisinger, D.; Winogrodzki, T.; Klinger, B.; Schnieke, A. and Rieblinger, B. (2023). Genome Editing in Pigs. Methods Mol. Biol., 2631(1):393-417.

Ruan, J. L.; Liang, S. S.; Pan, J. P.; Chen, Z. Q. and Teng, X. M. (2023). Artificial oocyte activation with Ca^{2+} ionophore improves reproductive outcomes in patients with fertilization failure and poor embryo development in previous ICSI cycles. Front. Endocrinol., 14: 1-10.

Saravanabavan, S. and Rangan, G. K. (2023). Culture of Three-Dimensional Madin–Darby Canine Kidney (MDCK) Cysts for In Vitro Drug Testing in Polycystic Kidney Disease. Methods Mol Biol., 2664(1):135-144.

Sugaya, S. (2010). Pregnancy following calcium ionophore oocyte activation in an oligozoospermia patient with repeated failure of fertilization after ICSI. Clin. Exp. Obstet. Gynecol., 37(4):261-262.

Sun, B. and Yeh, J. (2021). Calcium oscillatory patterns and oocyte activation during fertilization: a possible mechanism for total fertilization failure (TFF) in human in vitro fertilization? Reproductive Sciences, 28(3): 639-648.

Swidan, K.; EL Sherbiny, G.; Mansour, A.; El-Haw, M.; Askar, A. and El-Badry, M. (2020). Antibacterial evaluation of Punica granatum as therapeutic agents against multi drug resistance bacteria isolated from infertile male's semen. Al-Azhar Int. Med. J., 5(1):125-132.

Tang, M.; Boel, A.; Castelluccio, N.; Cardona Barberán, A.; Christodoulaki, A.; Bekaert, B. and Heindryckx, B. (2022). Human germline nuclear transfer to overcome mitochondrial disease and failed fertilization after ICSI. J. Assisted Reproduction and Genetics, 39(19): 609-618.

Wang, L.; Wang, J.; Zhang, Y.; Qian, C.; Wang, X.; Bai, J. and Ai, A. (2023). Analysis of euploidy rates in preimplantation genetic testing for aneuploidy cycles with progestin-primed versus GnRH agonist/antagonist protocol. European J. Med. Res., 28(1): 1-9.

World Health Organization, (2010). WHO laboratory manual for the examination of human semen and

sperm-cervical mucus interaction. Cambridge University Press.

Yu, J.; Li, B.; Li, H.; Li, Q.; Nai, Z.; Deng, B. and Li, Y. (2023). Comparison of uterine, endometrial and subendometrial blood flows in predicting pregnancy outcomes between fresh and frozen-thawed embryo transfer after GnRH antagonist protocol: a retrospective cohort study. J. Obstet. Gynecol., 43(1):1-8.

Zaretsky, D.V. and Zaretskaia, M. (2020). Degradation products of amyloid protein: are they the culprits? Current Alzheimer Res., 17(11): 869-880.

Zhang, F.; Zhang, H.; Du, H.; Li, X.; Jin, H. and Li, G. (2023a). Application value of early-follicular phase long-acting gonadotropin-releasing hormone agonist long protocol in patients with resistant ovary syndrome. BMC Pregnancy and Childbirth, 23(1):1-9.

Zhang, X.; Hu, C. and Wu, L. (2023b). Advances in the study of genetic factors and clinical interventions for fertilization failure. J. Assisted Reproduction and Genetics,1(19): 1-19.

Zhu, X.; Tian, T.; Jiesisibieke, D.; Fang, S.; Zhang, N.; Ma, J. and Yang, R. (2023). Clinical outcome of different embryo transfer strategies after late rescue ICSI procedure: a 10-year total fertilisation failure cohort study. BMC Pregnancy and Childbirth, 23(1):1-10.

"تنشيط البويضات البشرية بعد فشل عملية التخصيب بالحقن المجهري"

أسامة محمد بدر[1]*، محمد شحاته عبد العال[2]، إيمان أنور حسن[2] ، هدي جمال الدين محمدي[1]

1- قسم البيوتكنولوجيا الحيوانية، معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية، جامعة مدينة السادات.
2- المركز الدولي الإسلامي للدراسات والبحوث السكانية جامعة الأزهر.
***البريد الإلكتروني للباحث الرئيسي: osama.badr@gebri.usc.edu.eg**

الملخص

العقم مشكلة رئيسية تؤثر على الفرد والمجتمع. هدفت الدراسة الحالية هو تقييم كفاءة طرق تنشيط البويضات المساعدة باستخدام الأيونوميسين و 7 ٪ إيثانول في تحسين نتائج الحقن المجهري في حالات فشل الحقن المجهري المتكرر بما في ذلك العقم الشديد لعامل الذكور وفشل الإخصاب السابق وفشل زرع الأجنة. الإخصاب الطبى المساعد هو استعمال التكنولوجيا الحديثة لعلاج حالات العقم،عملية الحقن المجهرى هى أحدث الطرق فى الإخصاب الطبى المساعد وهى تعنى حقن حيوان منوى واحد داخل سيتوبلازم البويضة وقد بينت عدة تقارير أن غالبية الأزواج الذين كانوا يعانون من الإخفاق المتكرر للحقن المجهري وقد تمت الإستفادة من تطبيق الحقن المجهري جنبا إلى جنب مع تنشيط البويضات المساعد. تنشيط البويضات هو سلسلة من الأحداث اللتي تجعل البويضة المخصبة جاهزة لبدأ الإنقسام وتكوين الجنين. ولذا كان الهدف من هذه الدراسة هو دراسة تأثير التنشيط المساعد للبويضات بواسطة ايونوميسين الكالسيوم و7% ايثانول في حالات الإخفاق المتكرر للحقن المجهري (حالات فشل الإخصاب وحالات ضعف وتشوهات الحيوانات المنوية الشديد). سجلت كلتا الطريقتين المستخدمتين في هذه الدراسة تأثيرا عالي الكفاءة على مدة العقم، والبويضات الناضجة والمخصبة، ومعدلات الإخصاب، ومعدلات الانقسام، ومعدلات الكيسة الأريمية، ودرجات الأجنة ومعدلات الحمل. كان الأيونوميسين أفضل بكثير من 7 ٪ إيثانول في علاج المرضى الذين يعانون من فشل الإخصاب بالحقن المجهري.