Original Article

Effect of Calcium Ionophore on the Embryonic Outcome of the Intracytoplasmic Sperm Injection in Female Patients with Bad Quality Oocytes: Prospective -Histomorphometric Study

Aya Abdrabo Baraka[1], Kawther A. Hafez[1], Hatem H. R. Elgamal[2], Ahmed M. Desouky[2] and Marwa A. Fetouh[1]

[1]Department of Anatomy and Embryology, [2]Department of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, Egypt

ABSTRACT

Intoduction: Fertilization failure is the most common problem in assisted reproductive technology. In incident of ovum and/or spermatozoa correlated fertilization difficulties after intracytoplasmic sperm injection, approaches of modified ICSI techniques, can be done using Ca 2+ ionophore.

The objective of the research: Effectiveness of Ca 2+ ionophore was studied on the embryonic outcome in female patients with bad quality oocytes while her partner has normal semen analysis.

Material and Methods: Thirty-eight couples have undergone ICSI, with female age ranging between 40 to 45 years old. The retrieved oocytes were subdivided into 4 groups; good quality oocytes (IA), good quality oocytes with adding Ca 2+ ionophore treatment (IIA) , bad quality oocytes (IB), in addition, bad quality oocytes with adding Ca 2+ ionophore treatment (IIB), all were inserted by the corresponding sperms. Examination of the embryos was done on day two after fertilization, cleavage stage, and blastula stage.

Results: Treated good quality oocytes showed fertilization of 92%of the oocytes compared to 68% of the untreated good quality oocytes. Treated bad quality oocytes showed fertilization of 85% of the oocytes compared to 65% of the untreated bad quality oocytes. The quality of embryos was enhanced on day two after fertilization, day three (cleavage stage), and day five (blastula stage) in both treated groups than in the untreated ones.

Conclusion : The activated oocytes resulted in better fertilization and embryonic development. Therefore, ICSI combined with artificial oocyte activation using Ca 2+ ionophore is useful in mid-age female patients with good and bad quality oocytes regardless the cause.

Received: 14 Novamber 2022, **Accepted:** 19 December 2022

Key Words: Ca 2+ ionophore; bad quality oocytes good quality oocytes; ICSI; Infertility.

Corresponding Author: Aya Abdrabo Baraka, MSc, Department of Anatomy and Embryology, Faculty of Medicine, Ain Shams University, Egypt, **Tel.**: +2011 44982 096 - +2010 1433 3883, **E-mail:** dr.ayabaraka@med.asu.edu.eg

ISSN: 1110-0559, Vol. 47, No. 1

INTRODUCTION

Infertility is demarcated by means of the incapability to get pregnancy normally without any artificial techniques after at minimum one year of regular unguarded intercourse. In many cases, sterility is a grade of subfertility in which 1of each 7 couples necessities a specialist support to conceive. Infertility is classified into primary and secondary. Primary infertility is when the couple don't have previous gravidities; and secondary infertility is when the couple has conceived previously full term infant, as the pregnancy may not have progressed in cases of abortion and ectopic pregnancy[1]

The chance of getting pregnant coincidentally depends on the length of the sexual connection for both partners, the occurrence of coitus, and the age of the couple. Normal, young couples have a 25% probability to becoming pregnant after one month of unsupervised sexual activity; 70% of couples become pregnant by six months; and 90% of couples become pregnant by one year. After 1.5 or 2 years, only 5% of partners will become pregnant[2].

The reason of infertility is equally with both parties. Male factor, ovulatory insufficiency, or tubal-peritoneal illness are the three main reasons of infertility for the majority of infertile couples[3].

Intracytoplasmic sperm injection (ICSI) brings an operative methods in the field of assisted reproductive technology (ART)[4]. At present, it is one of the most important and effective handling methodologies which is used by infertility treatment centers. ICSI revolutionised assisted reproductive technology, and today it is the key treatment option for severe male factor infertility. This procedure involves inseminating sperm right into the egg. The most frequent challenge in ART is fertilization failure. The main reasons why conventional in *vitro* fertilization (IVF) fails include abnormalities in the semen analysis, such as abnormal sperm morphology, extremely low sperm counts, and impaired sperm motility. Because sperm motility and the release of chemotactic factors are key components of IVF, ICSI is recommended in cases of sperm motility impairment[5].

 DOI: 10.21608/ejh.2022.174130.1811

Many doctors recommend either ICSI or IVF to patients with tubal aspect infertility[6].

In the meantime, sperm and oocyte collaboration defined as fertilization process through which activation of the arrested oocyte in the second metaphase was done by the sperm[7]. This process is characterized by well-arranged structural, biological and chemical variations which are very important for the normal sequence of the growth. These include first dissemination of the cumulus and penetration of oocyte zona by sperm, then blending with the oocyte cell membrane[7]. These events are important for oocyte activation, which is characterized by exocytosis of cortical granules. The granules are essential for the zona pellucida toughness and blockage of the polyspermy followed by meiosis achievement with also ejection 2nd polar body and formation of spermatozoon and oocyte pronuclear (2PN) stage, pronuclear combination then start the embryo cell sequences[8,9].

The physiological mediator of oocyte activation in *vivo* has been identified as sperm-borne phospholipase C z (PLCZ)[10]. Normally, this factor enters the ooplasm and cleaves membrane-bound diacylglycerol complying with phosphatidylinositol biphosphate (PIP2), which starts the zonal response, and inositol triphosphate (IP3). Following this, IP3 binds to its receptor at the endoplasmic reticulum, causing calcium release and resulting in intracellular calcium oscillations[11,12].

The ensuing Ca2+ exhibits oscillating properties. In the absolute absence of Ca2+ fluctuations, any deficiency in these essential biochemical components (such as Plcz, PIP2, and IP 3) will spontaneously result in a decrease in intracellular calcium. By artificially boosting calcium in the oocyte and causing oocyte stimulation, this obvious downside can be compensated for. After ICSI, procedures like electrical oocyte activation[13,14,15] or modified ICSI techniques[16,17] have been successfully used to rescue oocyte activation in the case of oocyte and/or sperm-related fertilization issues. A variety of chemical mediators with calcium ionophores, such as ionomycin or calcimycin (A23187), are used to carry out this artificial operation[18].

THE AIM OF THE WORK

Was to evaluate the effect of Ca 2+ ionophore on the embryonic developmental outcome in female patients with good and bad quality oocytes as regards the fertilization, cleavage phase (2^{nd} and 3^{rd} day after fertilization) , then blastula stage (day 5 after fertilization).

MATERIAL AND METHODS

This prospective human study took place in the Laboratory of Fertilization & ICSI, Faculty of Medicine – Ain Shams University, Cairo, Egypt on 38 couples.

The design was approved, and the study was performed in agreement with the guidelines of the Medical Ethical Committee of Ain-Shams University.

Before starting the treatment, all couples were asked to sign a written consent in which they agreed to disclose the results of their own cycles for study purposes.

Females aged 40-45, of either unexplained infertility, or having a direct tubal causes as bilateral tubal blockage - peri tubal adhesions- bilateral hydrosalpinx , endometriosis or polycystic ovary syndrome were participating. Besides the inclusion of male partners with unexplained infertility and normal analysis of semen as regard WHO recommendations (2020)[19].

Semen volume: >1.4 ml per ejaculate

PH: >7.2

Sperm concentration: >16 million spermatozoa per ml

Total sperm count: >39 million spermatozoa per ejaculate

Total motility: >42% motile (percentage of progressive motility and non-progressive motility).

Progressive motility: > 30%

Vitality: 54% or more live spermatozoa,

Sperm morphology (percentage of normal forms): 4% or more.

Patients with uterine malformations or with severe male factor of infertility as globospermia and testicular biopsy samples (TESE) in which Ca 2+ ionophore was routinely used in ICSI cycles were excluded.

Study Design

Study groups

The oocytes were retrieved from thirty – eight couples who undergone ICSI cycles and divided into four groups , all were fertilized with the corresponding normal semen.

Ovarian stimulation[20]

All female partners were undergoing two ovarian stimulation protocols; either long or short antagonist protocols, the decision of that owed to the gynecologist.

A long protocol included down-regulation inhibition of the GNRH-agonist (Decapeptyl 0.1 mg, subcutaneous injection, Ferring Pharmaceuticals Ltd, India) with folic acid prescription in the mid luteal stage of the preceding phase. The recombinant FSH "Gonal-F® intramuscular injection- Geneva- Serono- Switzerland" was prescribed on the 2^{nd} or 3rd day of the cycle , with adding menopausal human gonadotropin "Menogon® intramuscular injection - Ferring Pharmaceuticals- Wittland- Germany" according to the follicular diameter and levels of hormonal profile.

A short antagonist protocol included Cetrortid 0.25mg subcutaneous injection (Merck Serono, Vienna, Austria) accompanied with administration of menopausal human gonadotropin "Menogon® intramuscular injection - Ferring Pharmaceuticals- Wittland- Germany" according to the follicular diameter and levels of hormonal profile.

Ovulation was identified when three ovarian follicles measuring 18 mm or greater were found. Oocyte collection was scheduled 36 hours after ovulation , which was induced with 10000 IU b-Human Chorionic Gonadotropin (HCG) (Ovitrelle® subcutaneous injection, Serono, Geneva, Switzerland)[20].

Oocyte pickup[20]

Trans-vaginal aspiration of follicles was happened under general anesthesia which was planned after recombinant HCG administration by 36 hour. Oocytes were retrieved and then incubated for an hour at 37 °C and 6% CO2 in culture medium (G-MOPSTM-V3-Plus, *Vitro*life, Kungsbacka, Sweden) covered with mineral oil (OvoilTM).

Quantity of cumulus oocyte complex (COC) retrieved from the female patients was 115 (COC).

Preparation of sperm samples[17]

By masturbation afterward 3–7 days of ejaculatory self-restraint ejaculated sperms were taken; then liquefaction on the laboratory temperature after that samples of sperm were prossesd by discontinuous-density-gradient-centrifugation or sperm swim/up procedure accompanied with sperm wash techniques .

Oocyte denudation[20]

After the cumulus cells from the collection (COC) were manually removed using finely drawn glass pasteur pipettes (Humagen Fertility Diagnostics, Charlottesville, Virginia, USA) under a stereomicroscope for 30 to 60 seconds, coronal cells were manually removed using HEPES-buffered standard containing 80 IU/ml hyaluronidase (Irvine Scientific, Santa Ana, USA).

Examination of the denuded oocytes were done for development and integrity. MII oocytes which only had expulsed their 1st polar body were inserted with spermatozoa with exclusion of all germinal vesicle GV and MI oocytes.

The number of oocytes after denudation was 92 MII oocytes and according to the quality of oocytes: the zona pellucida, cytoplasm, perivitelline space, the oocytes were divided into good quality oocytes and bad quality oocytes by using the inverted microscope (Eclipse TE 300, Nikon®, Tokyo, Japan). The good quality oocytes were detected being rounded in shape with intact zona pellucida, visible PB intact in the PVS and clear cytoplasm[21].

The oocytes were divided after examination into 48 oocytes MII good quality and 44 oocytes MII bad quality, further subdivided into four groups:

Group IA " control group" : 24 good quality oocytes were injected with the corresponding sperms without Ca 2+ ionophore.

Group II A: 24 good quality oocytes were injected with the corresponding sperms with Ca 2+ ionophore.

Group IB: 22 bad quality oocytes were injected with the corresponding sperms without Ca 2+ ionophore.

Group IIB: 22 bad quality oocytes were injected with the corresponding sperms with Ca 2+ ionophore.

ICSI technique and treatment using calcium ionophore[21]

Oocytes were individually placed in 4 l droplets of buffered media (G-MopsTM,V3-Plus-*vitro*life-Sweden) for ICSI. Spermatozoa were kept in a central 4 microliter droplet of poly vinyl pyrolidone solution ("PVP- Irvine Scientific-USA") in a 50 x 40 mm glass culture dish (willco-dish®- New Jersey- USA) protected with heated mineral oil ("OvoilTM- *vitro*life- Sweden").

The inverted microscope (Eclipse TE 300-Nikon®-Tokyo-Japan) heated stage (37OC) was used for sperm inoculation 38 hours after recombinant HCG triggers.

Following sperm injection into the appropriate oocytes, the previously chosen groups were incubated for 30 minutes at 37°C and 6% CO2 in culture medium containing 5 Mmol/l of the calcium ionophore A23187 (4-bromo calcium ionophore A23187, Sigma B7272, EUA). The injected oocytes were then washed and incubated in cultured medium (G-1TM-V3-Plus *Vitro*life in Sweden) at 37°C and 6% CO2.

Cell cycle embryo assessment[22]

Fertilization was assessed 18 h after ICSI, as the normal fertilization was confirmed when two clearly distinct pronuclei were existing.

Assessment of embryo quality was performed at day two, day three and day five after fertilization using the inverted microscope (Eclipse TE 300, Nikon®, Tokyo, Japan) according to the recording of the following parameters:

i. The number of cells according to embryo age

ii. The percentage of fragmentation

iii. Difference in blastomere symmetry

iv. The existence of multinucleation

Using such a grading system of Gardner scoring , it was determined that when two high-scoring blastocysts (4AA and 3AA; expanded blastocoel with compacted ICM and cohesive trophectoderm epithelium) were present, this indicated good quality embryos[22].

The criteria of good quality embryo (>3AA) according to Gardner classification[22]

A. The blastocoel cavity is full

B. the intracellular mass (ICM) are numerous and tightly packed

C. The trophectoderm cells are numerous and cohesive.

D. As regards the embryo transfer, it was done on day three or in day five after fertilization according to the quality of the embryo.

Morphometric study

After collection of data, calculation of percentage of development of good quality embryos in day two, cleavage stage, and blastula stage was done according to the previous mentioned criteria using Chi-square technique.

Comparison of the embryonic outcome of treated versus non-treated groups was done.

RESULTS

Histological results

Examination of human unstained denuded oocytes of group IA & IIA, detected oocytes with clear cytoplasm, normal zona pellucida and PB appeared in the PVS with normal size, few number of the oocytes showed minute cavitation in the cytoplasm (Figure 1).

The oocytes of group IB & IIB showed dark granular cytoplasm, dark zona pellucida and sub-zonal debris. Fragmented PB was noticed in some of oocytes (Figure 1).

Examination of human embryos day two of group IA and IIA showed four symmetrical cells with no fragmentation or multinucleation (Figure 2).

In group IB, some oocytes showed no fertilization and embryos appeared with three asymmetrical cells (Figure 2).

In group (IIB), embryos revealed three or four symmetrical cells with no multinucleation or fragmentation (Figure 2).

Examination of human embryos day three of the four groups revealed embryos with seven to eight symmetrical cells without fragmentation or multinucleation in group IA & group IIA. However, few numbers of embryos appeared with four symmetrical cells (Figure 3).

In group IB; embryos showed seven to eight asymmetrical cells with multinucleation, while in group IIB; most of the embryos revealed seven to eight symmetrical cells with fragmentation and no multinucleation. However, few number of embryos showed five asymmetrical cells with multinucleation (Figure 3).

Examination of human embryos day five of the four groups showed full blastocoel cavity in group IA & IIA. The ICM was composed of many tightly packed to mild loosely arranged cells and the trophectoderm cells were numerous and cohesive. Few embryos appeared compact with many fragmentation (Figure 4).

In group (IB); embryos showed empty blastocoel cavities, the ICM was composed of many loosely arranged cells while numerous embryos appeared compact with many fragmentation (Figure 4).

In group (IIB); embryos showed full blastocoel cavity, the ICM was composed of few loosely arranged cells, the trophectoderm cells were numerous and cohesive, number of embryos showed cavitating morula with no fragmentation and little number appeared compact with many fragmentation (Figure 4).

Morphometric results

The fertilization rate in the good quality oocyte groups was 68% in group IA compared to 92% in group IIA. While in the bad quality oocyte groups, it was 65% in group IB compared to 85% in group IIB (Table 1, Bar Chart 1).

As regards the quality of embryos at the cleavage stage (day 2& day 3); the good quality embryos (grade A) which appeared as symmetrical cells in shape with no fragmentation or less than 20% and no multinucleation were 68% in group IA compared to 92% in group IIA. While in group IB the good quality embryos (grade A) were 66% compared to 89% in group IIB (Table 2, Bar Chart 2).

The percentage of bad quality embryos (grade B) in group IA was 32% compared to their percentage in group IIA was 8%, while in group IB, the percentage of bad quality embryos was 34%comapred to their percentage in group IIB was 11% (Table 2, Bar Chart 2).

At the blastula stage, the good quality embryos (>3AA) were 47% in group IA compared to 91% in group IIA, but in the bad quality oocyte groups, the good quality embryos (> 3AA) were 25% in group IB compared to 89% in group IIB (Table 2, Bar Chart 2).

As regards the compact embryos in the blastula stage, the bad quality embryos were 53% in group IA compared to 9% in group IIA, while their percentage were 75% in group IB compared to 11% in group IIB (Table 2, Bar Chart2).

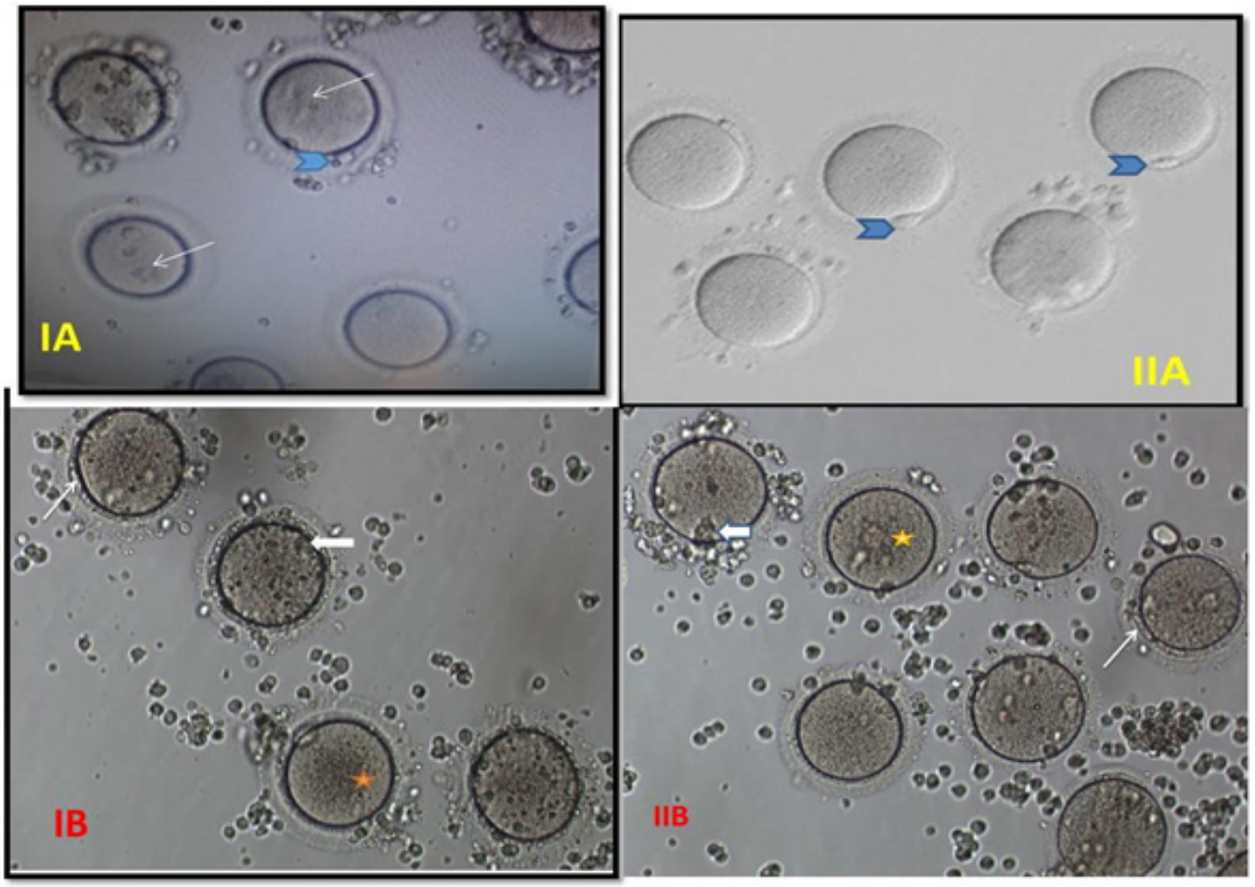


Fig. 1: Photomicrographs of human unstained denuded oocytes of group (IA & IIA) showing clear cytoplasm, normal zona pellucida and PB appears in the PVS with normal size (arrowhead). Notice that one of the oocytes of IA shows minute cavitation in the cytoplasm (arrow). The oocytes of group (IB&IIB) show dark granular cytoplasm (*), dark zona pellucida, sub-zonal debris (thin arrow) and one oocyte with fragmented PB (thick arrow). Inverted microscope x40

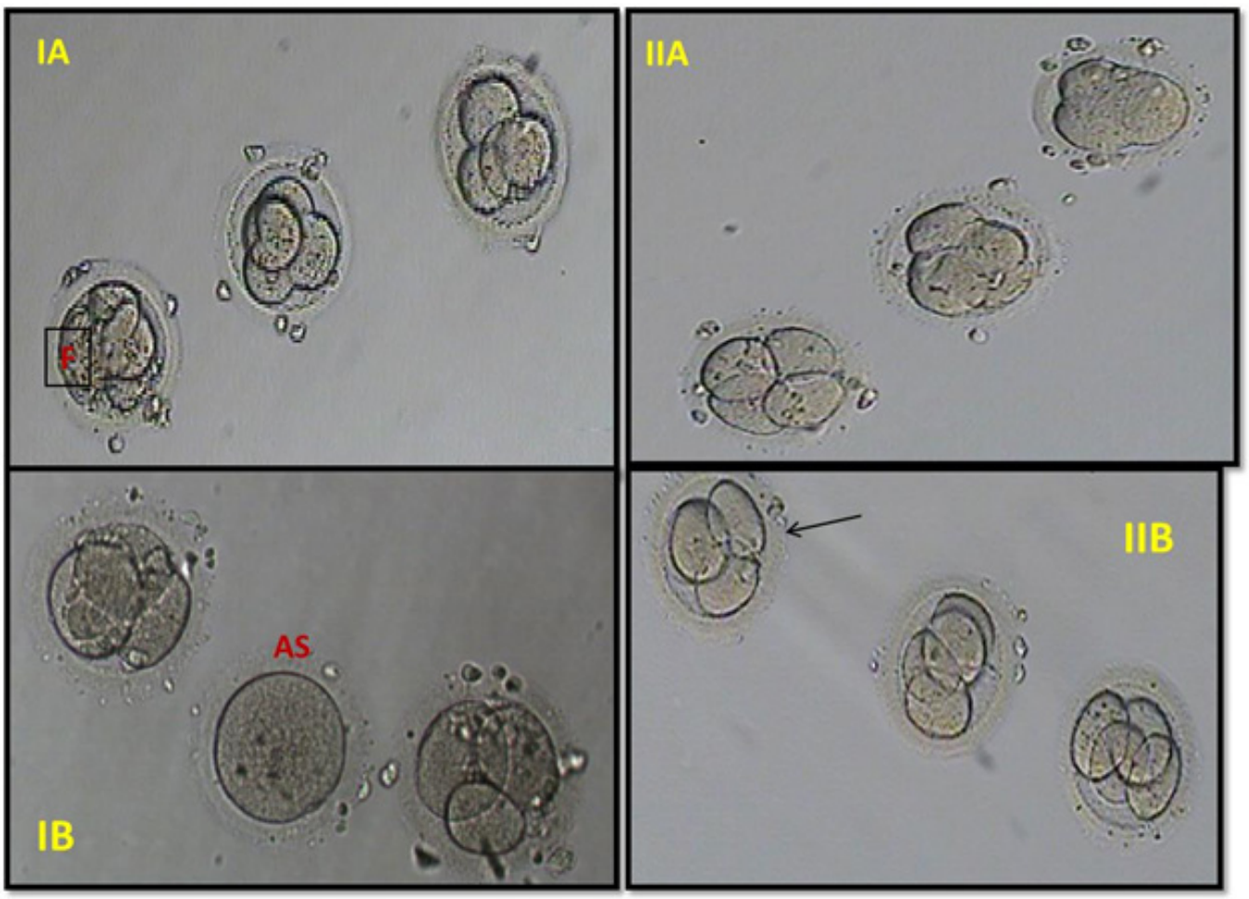


Fig. 2: Photomicrographs of living human embryos on day two of the four groups showing embryos in group IA and IIA with four symmetrical cells. Notice the presence of fragmentation (F) in an embryo of group IA. In group IB, an oocyte (AS) shows no fertilization and the other embryos show three asymmetrical cells. In group (IIB), two embryos reveal four symmetrical cells and an embryo (arrow) with three symmetrical cells. Inverted microscope, x40

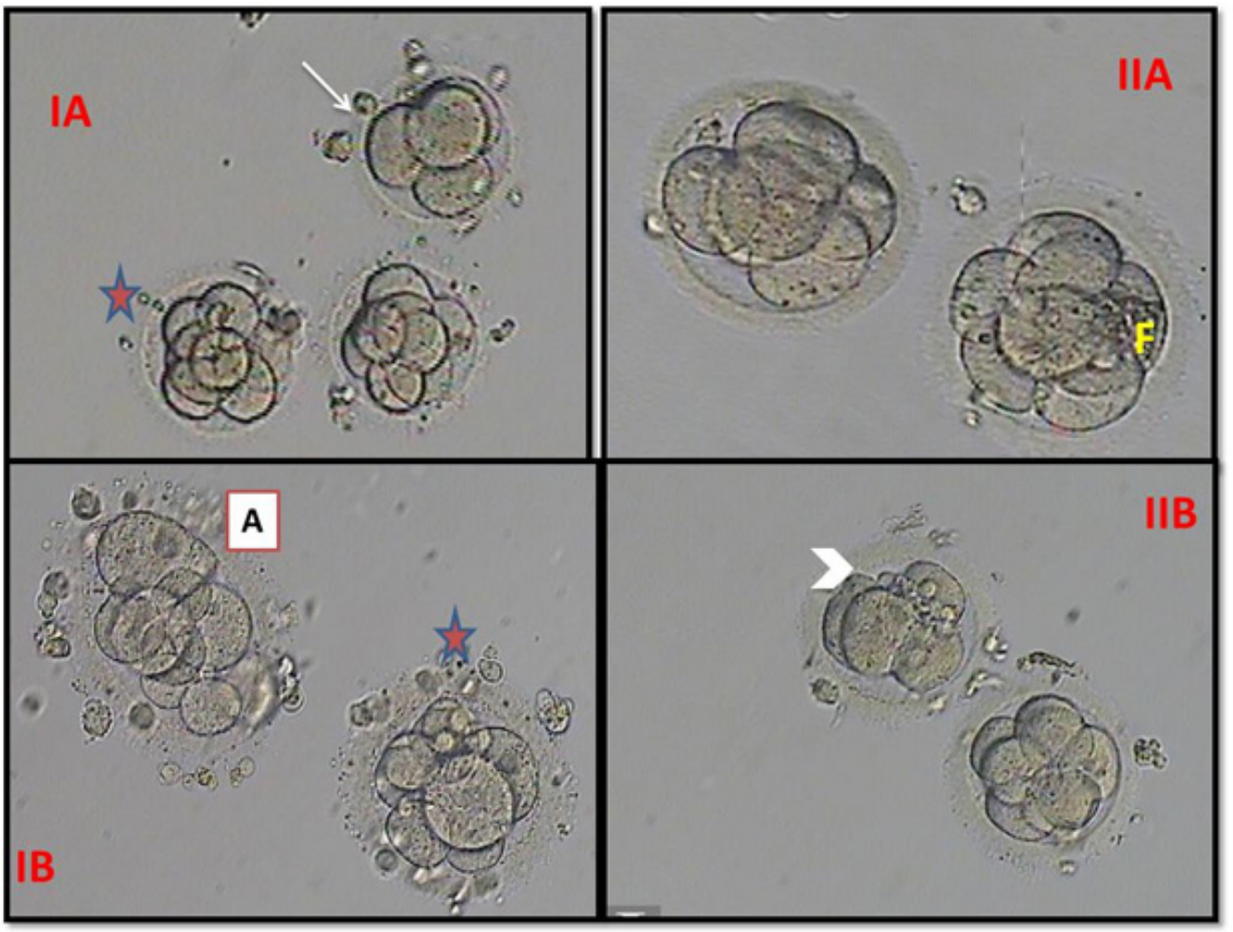


Fig. 3: Photomicrographs of living human embryos on day three of the four groups showing embryos in group IA with symmetrical cells and no multinucleation. Notice an embryo (arrow) with four cells, another embryo (*) with seven cells, and a third embryo with eight cells. In group IIA; one embryo reveal eight symmetrical cells with fragmentation (F) and the other embryo shows seven symmetrical cells.
In group IB; the embryo (A) shows eight asymmetrical cells and the embryo (*) shows seven asymmetrical cells with multinucleation.
In group IIB; one embryo (arrow head) reveals five asymmetrical cells with multinucleation and the other embryo shows eight symmetrical cells. Inverted microscope, x40

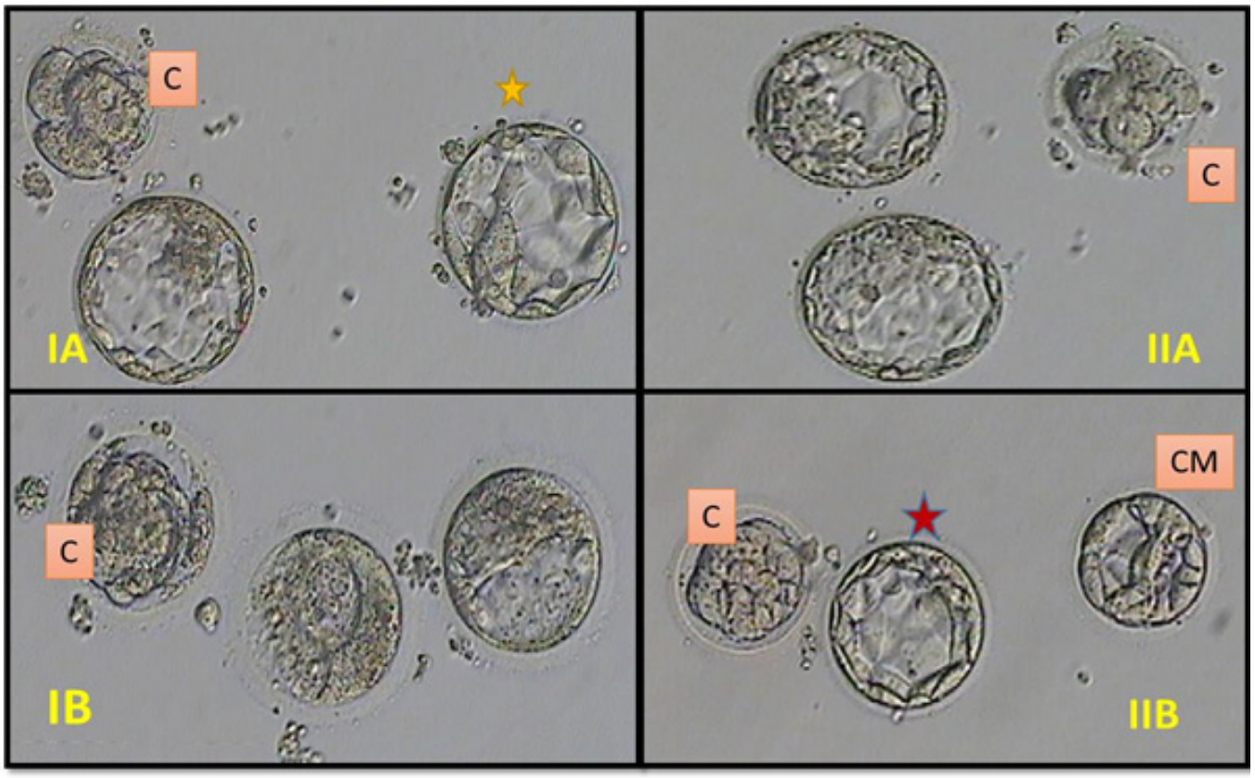


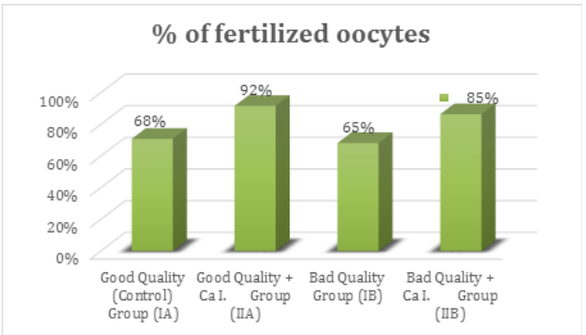
Fig. 4: Photomicrographs of living human embryos on day five of the four groups showing in group IA; a compact embryo (C) with many fragmentation and another embryo (*) with an empty blastocoel cavity, the ICM is composed of many loosely arranged cells. The third embryo shows a full blastocoel cavity, the ICM is composed of many tightly packed cells, and the trophectoderm cells are numerous and cohesive. In group (IIA); the two embryos presenting the ICM is composed of many loosely arranged cells, while an embryo (C) is compact with fragmentation. In group (IB); the two embryos showing partially empty blastocoel cavities, the ICM is composed of many loosely arranged cells and embryo (C) presenting compact embryo with many fragmentation. In group (IIB); the embryo (C) is compact with many fragmentation, an embryo (*) showing a full blastocoel cavity, the ICM is composed of few loosely arranged cells, the trophectoderm cells are numerous and cohesive, and embryo (CM) presenting cavitating morula with no fragmentation. Inverted microscope, x40

Table 1: percentage of fertilized oocytes in each group

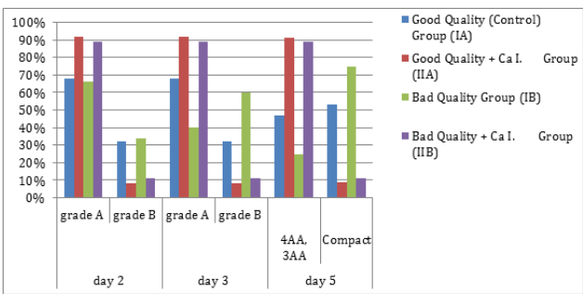
	Good Quality (Control) Group (IA)	Good Quality + Ca I Group (IIA)	Bad Quality Group (IB)	Bad Quality + Ca I. Group (IIB)
% of fertilized oocytes	68%	92%	65%	85%

Table 2: percentage of good and bad quality embryos in day 2, 3, 5 in each group

		Good Quality (Control) Group (IA)	Good Quality + Ca I Group (IIA)	Bad Quality Group (IB)	Bad Quality + Ca I. Group (IIB)
Day 2 (4 cell stage) cleavage stage	grade A	68%	92%	66%	89%
	grade B	32%	8%	34%	11%
Day 3(8 cell stage) cleavage stage	grade A	68%	92%	40%	89%
	grade B	32%	8%	60%	11%
Day 5 (blastula stage)	4AA, 3AA	47%	91%	25%	89%
	Compact	53%	9%	75%	11%



Bar Chart 1: Percentage of fertilized oocytes in each group.



Bar Chart 2: Percentage of good and bad quality embryos in day two, three, five in each group.

DISCUSSION

One of the most important techniques in reproductive assisted techniques is ICSI through it one spermatozoa is injected directly into the cytoplasm of oocyte then detection of fertilization[23].

Many studies acknowledged the influence of Ca 2+ ionophore in cases of severe male aspect and also in cases of preceding fertilization failure or embryo cleavage arrest[20], but its effectiveness role in the case of females with bad feature oocytes is still debated, which was the scope of the current research.

In the current study, the effect of Ca 2+ ionophore was studied on the embryonic outcome in cases of female patients aged 40-45 years with good and bad quality oocytes while their partners have normal semen analysis.

The oocytes held in follicles in the ovarian cortex (i.e., the ovarian reserve) decline with age in both quantity and quality[24]. The age-related decline in fertility also depends on differences in endocrine and endometrial function and is closely correlated with oocyte ageing[25].

In the present study, bad quality oocytes (group IB, IIB) showed fragile oocytes, wide perivitelline space, cavitation in the cytoplasm, dark granular cytoplasm, and fragmented polar body. All these changes affect the fertilization and quality of embryos which was diagnosed by previous studies[26].

These cytoplasmic and extra cytoplasmic defects were accepted in several preceding studies as a sign of fertilization failure and affect the quality of embryos. The presence of an atypical first polar body (fragmented and or large) in the metaphase II oocytes was related to a decline in the fertilization frequency or formation of aneuploidy oocyte. Disintegrated polar bodies have been hypothesised to represent an asynchrony between nuclear and cytoplasmic evolution. The pronuclear morphology and fertilization rate of the injected oocytes were significantly impacted by the wide perivitelline gap[27].

Oocyte triggering is described by a surge followed by diffusion of intracellular Ca2+ oscillations. Sperms are the ordinary provocation accountable for encouraging Ca2+ waves , which lead to a multifarious sequence of occasions that convert the arrested MII ovum into a pronuclear stage which is known as (oocyte stimulation)[27].

Oocyte stimulation can be done artificially using multiple stimuli variation which may be mechanical-electrical and also chemical ; leading to intracellular Ca2+ level increase[28].

The best extensively used technique for oocyte activation after ICSI is chemical oocyte stimulation[29]. The activation can also be done using multiple variation of chemical mediators as : ethanol 7%[30], strontium chloride, phorbol ester[31], thimerosal[32], and Ca2+ ionophore[33,34,35,36].

Wide-ranging studies in mammals[37,38] have been approved that oocyte stimulation, which was presented by increase the incidence of Ca2+ waves intra cellular accompanied with improvement of embryonic cell cycle[39].

Furthermore; Ca2+ indications started equally in fertilization and in early post-fertilization, which are linked to supplementary embryo growth[40].

Therefore, inadequate Ca 2+ signaling can end in developing capture of the newly oocytes which were fertilized. Thus in case of 2PN-arrested oocytes, embryo improvement needs to be generated with effective Ca 2+ therapy[41].

In the current research, the influence of artificial oocyte activation AOA using Ca2+ ionophore on the embryo growth was evaluated in partners with history of unexplained infertility, ovarian and tubal factors which mainly affect the quality of oocytes. Patients in the current study were underling the ICSI trial for the first time.

Based on the previous published study ; on the starring role of Ca2+ in embryonic cell cycle growth, Ca2+ signals started in the stimulated ovum may have been unsatisfactory, leading to sub-optimal oocyte activation sequences , that properly be related to additional cleavage disappointment[42]. Previous studies supposed that oocyte actiavtion with Ca2+ ionophore ; might overcome the difficulty of insufficient signaling procedures , helping in advanced improvement[43,44].

In the current study, the Ca 2+ ionophore had a positive effect on the group IIB of poor quality oocytes, increasing

both the fertilization rate and the quality of the resulting embryos in comparison to group IB.

The fertilization rate percentage was 68% in the untreated good quality oocytes while it was 92% in the treated good quality oocytes . In bad quality oocytes, the fertilization rate increased from 65% in the untreated group to 85% in the treated group.

CA ionophore has formerly been described as a real management in circumstances of incomplete and also complete fertilization arrest after ICSI[42], resulting in enhanced fertilization, pregnancy, and healthy live births[43,44].

The quality of embryos was enhanced in both treated groups (group IIA, IIB) than in the untreated groups (group IA, IIB) on day two after fertilization, day three (cleavage stage), and day five (blastula stage).

In cleavage stage the percentage of good quality embryos in treated groups was 92% & 89%while in the untreated groups it was 68% & 66%.

Based on the previous studies , there was improvement in embryo quality in cases of AOA by Ca2+ ionophore during the cleavage and blastula stage even during the follow up of patients with increase the incidence of pregnancy rate[17,20].

Finally, there is still hope to be a mother and get a baby even if you are over forty or have bad quality oocytes, whatever the cause . Oocyte activation by Ca2+ ionophore should be mandatory in cases where it is needed.

CONCLUSION

It can be concluded that activated oocytes resulted in better fertilization and embryonic development. Therefore, ICSI combined with AOA using Ca2+ ionophore is useful in mid age female patients with bad quality oocytes regardless of the underlying cause.

HUMANRIGHTSSTATEMENTSANDINFORMED CONSENT

The entire experimental study was carried out in agreement with the regulations permitted by the Research Ethics Committee, Faculty of Medicine, Ain Shams University, which complied with polish legal necessities and EU directive 2010/63/EU of the European parliament and the council of September 22, 2010, as well as the national research council 2011 requirements. Ethical committee approval number: FMASU MD 91/2020 S I D: 354

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Anwar S, Anwar A. Infertility: A Review on Causes, Treatment and Management. Women's Health & Gynecology Journal. 2016; 2:1-5.
2. Kamel RM. Management of the infertile couple: an evidence based protocol. Reprodbiol Endocrinol. 2010; 8-21.
3. Kakarla N, Bradshaw K. Evaluation and Management of the Infertile. Glob. Libr. Women's med.: 2008; 1756-2228.
4. Tarlatzis BC, Bili H. Survey on intracytoplasmic sperm injection: report from the ESHRE ICSI Task Force.European society of human reproduction and embryology. Hum.Reprod.13. 1998;(Suppl1):165–177.
5. Andersen N, Goossens V, Gianaroli L, Felberbaum R, de Mouzon J, Nygren KG. Assisted reproductive technology in Europe, 2003.Results generated from European registers by ESHRE. Hum Reprod. 2007; 22(6):1513–1525.
6. Mahnaz A, Shahideh J,Mohammad R, Firouzeh G, Zahra Z. ICSI Outcome in Infertile Couples with Different Causes of Infertility: A Cross-Sectional Study. Int J fertile.Steril. 2013; 7(2): 88–95.
7. Williams CJ Signaling mechanisms of mammalian oocyte activation. Hum. Reprod. Update. 2002; 8:313-321.
8. Iwao Y Egg activation in physiological polyspermy. Reproduction. 2012; 144, 11–22.
9. Anifandis G ,Messini Ch , Dafopoulos K, Sotiriou S, Messinis I. Molecular and Cellular Mechanisms of Sperm-Oocyte Interactions Opinions Relative to in *Vitro* Fertilization (IVF). Int. J. Mol. Sci.2014; 15(7):12972-12997.
10. Saunders CM , Larman M G, Parrington J, Llewellyn J C, Royse J, Blayney LM, *et al*. PLC zeta: a sperm-specific trigger of Ca2+ oscillations in eggs and embryo development. Development. 2002; 129:3533–3544.
11. Berridge MJ. Inositol trisphosphate and calcium signalling mechanisms. Biochimbiophys Acta. 2009; 1793:933–940
12. Davina B, Minerva F, Lien D, Mina P, Vanessa T, Ilse De C ,*et al*. Assisted oocyte activation significantly increases fertilization and pregnancy outcome in patients with low and total failed fertilization after intracytoplasmic sperm injection:a 17-year retrospective study. Fertility and Sterlity.2019;15-282.
13. Yanagida K, Fujikura Y, Katayose H. The present status of artificial oocyte activation in assisted reproductive technology. Reprod. Med. Biol. 2008;7(3):133–142
14. Ebner T, Montag M, Van der K, Shebl O , Oppelt P, Hirchenhain J , *et al*. Live birth afterartificial oocyte activation using a ready-to-use ionophore: a pro spectivemulticentre study. Reprod. Biomed.2015;Online 30: 359–365.

15. Baltaci V, Ayvaz OU, Unsal E, Aktaş Y, Baltaci A, Turhan F ,etal. The effectiveness of intracytoplasmic sperm injection combined with piezoelectric stimulation in infertile couples with total fertilization failure. Fertil.Steril.2010; 94:900–904

16. Tesarik J, Rienzi L, Ubaldi F, Mendoza C, Greco E. Use of a modified intracytoplasmic sperm injection technique to overcome sperm-borne and oocyte-borne oocyte activation failures. Fertil.steril. 2002; 78:619–624.

17. Ebner T , Oppelt P , Wöber M , Staples P , Mayer R B , Sonnleitner U , *et al*. Treatment with Ca21 ionophore improves embryo development and outcome in cases with previous developmental problems: a prospective multi-center study. Human reproduction. 2014; Vol.30,No.1pp: 97- 102

18. Vanden M, Nikiforaki F, De Gheselle D, Dullaerts S, Van den Abbeel V, Gerris E, *et al*. Assisted oocyte activation is not beneficial for all patients with a suspected oocyte-related activation deficiency. Hum Reprod. 2012;27:1977–1984

19. World Health Organization Health problems, problem behaviours and risk factors Infertility Annex 2020; 3:47-49.

20. Darwish E, Magdi Y. A preliminary report of successful cleavage after calcium ionophore activation at ICSI in cases with previous arrest at the pronuclear stage. Reproductive biomedicine. 2015; Online 31:799–804.

21. Norozi-Hafshenjani M. Effect of assisted oocyte activation with calcium –ionphore and strontium chloride on in*vitro* ICSI outcomes.Iran J Basic. Med. Sci.2018; 21(11):1109-1117.

22. Gardner D., Weissman A., Howles C. and Shoham Z. Textbook of Assisted Reproductive Techniques, Blastocyst stage embryo:Vol.1&2;2017.

23. Palermo G, Joris H, Devroey P, Van Steirteghem, AC, *et al*. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet. 1992; 340: 17–18.

24. 2Klinkert ER. Clinical significance and management of poor response in IVF. Thesis Utrecht University. 2005.

25. Igarashi H, Takahashi T, Nagase S. Oocyte aging underlies female reproductive aging: biological mechanisms and therapeutic strategies. Reprod. Med. Biol. 2015; 14(4): 159–169.

26. De Santis L, Cino I, Rabellotti E. Polar body morphology and spindle imaging as predictors of oocyte quality. Reprod Biomed. 2005;Online11:36–42.

27. Nasr-Esfahani MH, Deemeh MR, Tavalaee M. Artificial oocyte activation and intracytoplasmic sperm Injection. Fertil.Steril.2010; 94: 520–526.

28. Alberio R, Zakhartchenko V, Motlik J , Wolf E. Mammalian oocyte activation: lessons from the sperm and implications for nuclear transfer. Int. J. Dev. Biol. 2001;45: 797–809.

29. Ebner T, Köster M, Shebl O, Moser M, Van der Ven H, Montag M, *et al*. Application of a ready-to-use calcium ionophore increases rates of fertilization and pregnancy in severe male factor infertility. Fertil. Steril. 2012; 98: 1432–1437.

30. Presicce GA, Yang X. Parthenogenetic development of bovine oocytes matured in *vitro* for 24 hr and activated by ethanol and cycloheximide. Mol. Reprod. Dev. 1994; 38:380–385.

31. Cuthbertson KS, Cobbold PH. Phorbol ester and sperm ac tivate mouse oocytes by inducing sustained oscillations in cell Ca2. Nature. 1985; 316: 541–542.

32. Fissore R, Robl JM. Sperm, inositol triphosphate, and thimerosal-induced intracellular Ca2 elevation in rabbit eggs. Dev.Biol.1993; 159: 122–130.

33. Méo SC, Yamazaki CR, FerreiraF, Perecin NZ, Saraiva CL, Garcia JM. Parthenogenetic activation of bovine oocytes using single and combined strontium, Ionomycin and 6-dimethylaminopurine treatments. Zygote.2007; 15: 295– 306.

34. Hosseini SM, Hajian M, Moulavi F, Shahverdi AH, Nasr-Esfahani MH. Optimized combined electrical-chemical parthenogenesis activation for in *vitro* matured bovine oocytes. Anim. Reprod. Sci. 2008; 108:122–133.

35. Santella L ,Dale B. Assisted yes, but where do we draw the line? Reprod.Biomed.2015;Online doi: 10.1016/j.rbmo.2015.06.013; <http://www.rbmojournal.com/article/S1472-6483%2815%2900309-0/fulltext>.

36. Van Blerkom J, Cohen J , Johnson MA. plea for caution and more research in the 'experimental' use of ionophores in ICSI. Reprod. Biomed. 2015; Online 30: 323–324.

37. Kline D, Kline JT. Repetitive calcium transients and the role of calcium in exocytosis and cell cycle activation in the mouse egg. Dev. Biol. 1992; 149: 80–89.

38. Keating T J, Cork R J, RobinsonK R. Intracellular free calcium oscillations in normal and cleavage-blocked embryos and artificially activated eggs of Xenopuslaevis. J. Cell Sci. 1994; 107: 2229– 2237.

39. Bos-Mikich A, Whittingham DG, Jones KT. Meiotic and mitotic Ca2+ oscillations affect cell composition in resulting blastocysts. Dev. Biol. 1997; 182, 172–179.

40. Plachot M, Selva J, Wolf JP, Bastit P, Mouzon J de. Consequences of oocyte dysmorphy on the fertilization rate and embryo development after intracytoplasmic sperm injection. A prospective multicenter study. Gynecol. Obstet. Fertil. 2002; 30: 772–779.

41. YuY, Saunders CM, Lai FA, Swann K. Preimplantation development of mouse oocytes activated by different levels of human phospholipase C zeta. Hum. Reprod. 2008; 23: 365–373.

42. Montag M, Ebner T. Activation Study Group. Clinical application of artificial oocyte activation: results of a prospective multicenter study. 2011;Abstracts of the 27th annual meeting of ESHRE, Stockholm, 265.

43. Kamiyama H, Shimizu T, Oki T, Asada T, Araki Y. Successful delivery following intracytoplasmic sperm in jection with calcium ionophore A23187 oocyte activation in a partially globozoospermic patient. Reprod. Med. Biol.2012; 11: 159–164.

44. Ebner T, Köster M, Shebl O, Moser M, Van der Ven H, Montag M, *et al*. Live birth after artificial oocyte activation using a ready-to-use ionophore: a prospective multicentre study. Reprod. Biomed.2015;Online 30: 359–365.

الملخص العربى

تأثير أيونوفور الكالسيوم على النتائج الجنينية لحقن الحيوانات المنوية داخل سيتوبلازم البويضات عند الإناث اللائي يعانين من البويضات ذات النوعية الرديئه : دراسة نسيجيه قياسيه

آيه عبد ربه علي بركه[١]، كوثر أحمد حافظ[١]، حاتم حسين رضوان الجمل[٢]، أحمد دسوقي[٢]، مروه عبد المنعم أبو الفتوح[١]

[١]قسم التشريح وعلم الأجنه، [٢]وحده الإخصاب المساعد مستشفي النساء التوليد، كليه الطب، جامعه عين شمس

المقدمة: فشل الإخصاب هو المشكلة الأكثر شيوعًا في تقنية المساعدة على الإنجاب. في حالة وجود صعوبات في الإخصاب مرتبطة بالبويضة و / أو الحيوانات المنوية بعد حقن الحيوانات المنوية داخل السيتوبلازم و يوجد طرق تقنيات الحقن المجهري المعدلة ، والتي يمكن إجراؤها باستخدام أيونوفور الكالسيوم.

الهدف من الدراسة: تمت دراسة فعالية ايونوفور الكالسيوم على النتيجة الجنينية لدى النساء المصابات ببويضات ذات نوعية رديئة بينما كان لدى شريكها تحليل طبيعي للسائل المنوي.

المنهجيه البحثيه: خضع ٣٨ زوجًا لعملية الحقن المجهري ، وتتراوح أعمار الإناث بين ٤٠ و ٤٥ عامًا

تم تقسيم البويضات المسترجعة إلى ٤ مجموعات ؛ البويضات ذات النوعية الجيدةIA)) والتى تمثل المجموعة الضابطة والبويضات ذات النوعية الجيدة مع علاج ايونوفور الكالسيوم (IIA)، البويضات ذات النوعية الرديئة (IB) والبويضات ذات النوعية الرديئة مع علاج ايونوفور الكالسيوم(IIB) ، تم حقنها جميعًا بالحيوانات المنوية المقابلة.

تم فحص الأجنة في اليوم الثاني بعد الإخصاب ومرحلة المورولا و مرحله تكوين الأريمه.

النتائج: أظهرت البويضات المعالجة ذات النوعية الجيدة إخصاب ٩٢٪ من البويضات مقارنة بـ ٦٨٪ من البويضات غير المعالجة ذات النوعية الجيدة وايضا أظهرت البويضات المعالجة ذات النوعية الرديئة إخصاب ٨٥٪ من البويضات مقارنة بـ ٦٥٪ من البويضات غير المعالجة ذات النوعية الرديئة.

تم تحسين جودة الأجنة في اليوم الثاني بعد الإخصاب ، واليوم الثالث (مرحلة المورولا) ، واليوم الخامس (مرحلة الأريمه) في كلتا المجموعتين المعالجتين أكثر من المجموعات غير المعالجة.

الاستنتاج النهائي: أدت البويضات المنشطة بايونوفور الكالسيوم إلى إخصاب أفضل وتطور جنيني افضل . لذلك ، فإن الحقن المجهري مع تنشيط البويضات الاصطناعية باستخدام ايونوفور الكالسيوم مفيد في النساء في منتصف العمر مع البويضات الجيدة والرديئة بغض النظر عن السبب.