Zeynep Kamil Med J 2022;53(4):170–175
DOI: 10.14744/zkmj.2022.14471

ORIGINAL ARTICLE

Calcium ionophore enhances blastocyst formation, embryo quality, and live birth delivery rates in patients with previous IVF failures

[1]Fırat TÜLEK
[2]Alper KAHRAMAN

[1]Department of Obstetrics and Gynecology, Memorial Ataşehir Hospital, İstanbul, Türkiye
[2]Department of Obstetrics and Gynecology, Haseki Training and Research Hospital, İstanbul, Türkiye

ORCID ID
FT : 0000-0003-1668-8746
AK : 0000-0002-1689-2782

ABSTRACT

Objective: The objective of the study was to evaluate the impact of artificial oocyte activation (AOA) with calcium ionophore (A23187) on *in vitro* fertilization outcomes of patients with previous history of cycle cancellations due to arrested embryos before formation of day 5 blastocysts.

Material and Methods: This retrospective study was conducted by evaluating records of patients that admitted for *in vitro* fertilization between 2013 and 2021. Patients with the previous history of developmental arrest in whole embryo cohort without fertilization failure were included in the study. Cycle outcomes of women with and without AOA were compared.

Results: A total of 347 women were found eligible and included in the study. One hundred and eighty-five of them were treated with AOA that constituted the study group and 162 women were included in the control group. Rate of good quality embryos (65.3% vs. 31.1%; p<0.001), implantation rates (0.33±0.41 vs. 0.22±0.39; p=0.003), clinical pregnancy rates (43.2% vs. 25.3%; p<0.001), and live birth delivery rates (40.5% vs. 21%; p<0.001) were higher in women treated with AOA with calcium ionophore in comparison to controls. Rate of multiple pregnancy was also found higher in calcium ionophore group (5.4% vs. 1.9%; p=0.01). Mean infant birth weights were similar among groups.

Conclusion: AOA with A23187 appears to improve progression to blastocyst rates, embryo quality, clinical pregnancy rates, and live birth delivery rates in women with a previous history of complete embryo development arrest before formation of day 5 blastocysts.

Keywords: A23187, artificial oocyte activation, calcium ionophore, *in vitro* fertilization, live birth rate.

Cite this article as: Tülek F, Kahraman A. Calcium ionophore enhances blastocyst formation, embryo quality, and live birth delivery rates in patients with previous IVF failures. Zeynep Kamil Med J 2022;53(4):170–175.

Received: April 16, 2022 **Revised:** June 04, 2022 **Accepted:** June 17, 2022 **Online:** November 04, 2022
Correspondence: Fırat TÜLEK, MD. Memorial Ataşehir Hastanesi, Kadın Hastalıkları ve Doğum Kliniği, İstanbul, Türkiye.
Tel: +90 530 608 48 74 **e-mail:** firattulek@yahoo.com
Zeynep Kamil Medical Journal published by Kare Publishing. Zeynep Kamil Tıp Dergisi, Kare Yayıncılık tarafından basılmıştır.

INTRODUCTION

Fresh transfers of day 5 blastocysts are associated with more favorable outcomes in comparison to transfer of earlier stages embryos regarding clinical pregnancy and live birth rates.[1] Hence, contemporarily, fresh transfer of day 5 blastocyst stage embryos is considered as the optimal choice of embryo transfer and widely used in reproductive health centers. However, some of the embryos in a cohort could not reach to blastocyst stage. Embryos those fail to demonstrate signs of mitotic division and cleaving activity for at least 24 h are considered "arrested" or "non-viable."[2] Blastomeres of arrested embryos may have been degenerated or lysed. In some cases, these arrested embryos could constitute the whole embryo cohort of a patient. Several factors may cause developmental embryo arrest including genetic variations, chromosomal abnormalities, changed pattern of protein expression, and alterations in metabolic pathways.[3] The European Society of Human Reproduction and Embryology indicated a benchmark value of progression to blastocyst rate as 60% in their survey.[4] That means approximately 40% of embryos would be arrested before forming a blastocyst. The prevalence of this phenomenon renders it a topic of interest for research to improve *in vitro* fertilization (IVF) outcomes.

Physiological intracellular calcium oscillations are crucial for oocyte fertilization and alteration of these oscillations leads to fertilization failures. The previous studies demonstrated that intracellular calcium fluxes also take part in post-fertilization mitotic events, particularly in the pronuclear zygote phase and cleaving embryo phase.[5] Signaling events in early post-fertilization period are known to influence future intracellular events, eventually embryo viability, and embryo quality.[5] Animal studies have shown that calcium chelators cease mitosis in cleavage stage embryos.[6] Furthermore, arrested human embryos fail to demonstrate normal sinusoidal calcium fluctuations preceding each mitotic division.[7] Calcium ionophore is a divalent cation ionophore that increases intracellular calcium concentrations and is widely studied as an adjuvant to increase fertilization rates in intracytoplasmic sperm injection (ICSI) cycles. Nevertheless, the effects of calcium ionophore on preventing embryo arrest are an understudied issue. In this study, we aim to evaluate the effects of artificial oocyte activation (AOA) with calcium ionophore on IVF outcomes in patients with previous IVF failures due to embryo developmental arrest.

MATERIAL AND METHODS

The study was conducted by retrospectively evaluating patient records that admitted to a university affiliated infertility center (Memorial Atasehir Hospital affiliated with Uskudar University) in Istanbul. Ethical approval for this study was obtained from the Ethical Committee of Uskudar University at 30/04/2021 (approval number: 61351342/April 2021-85).

Records of patients admitted to clinic between 2013 and 2021 that underwent ICSI were screened. Women with a history of previous IVF failure due to embryo developmental arrest before formation of day 5 blastocyst were included in the study. Women >45 years of age, cases with thyroid abnormalities or hyperprolactinemia, cases that underwent genetic screening, patients with severe male factor infertility, and patients with a history of previous cycle cancellation due to fertilization failure were excluded from the study. Only the outcomes of fresh embryo transfer cycles were assessed in this study.

Controlled ovarian stimulations were carried out by gonadotropin-releasing hormone (GnRH) antagonist protocols. Stimulation procedure was initiated on the 2nd menstrual cycle day. Recombinant follicle-stimulating hormone (rFSH, Gonal-F, and Merck Serono S.p.A), human menopausal gonadotropin (hMG, Merional, IBSA Institut Biochimique S.A, Menopur® Ferring Pharmaceuticals), or combination of recombinant luteinizing hormone and rFSH (Pergoveris, Merck Serono, SA) is used for ovarian stimulation on practitioner's preference. Gonadotropin doses are adjusted in accordance with routine patient monitoring by transvaginal ultrasound in each visit and serum hormone levels as required. GnRH antagonist agents (Cetrotide 0.25 mg, Pierre Fabre Medicament Production) are used to suppress premature LH peak starting from the day that the leading follicle has reached a diameter of 12–14 mm and continued to the day of oocyte maturation triggering. Once, at least one follicle has reached a diameter of 18 mm, final oocyte maturation is triggered either by concomitant injections of GnRH agonist of 0.2 mg triptorelin acetate, (Gonapeptyl, Ferring Pharmaceuticals) and 250 mcg recombinant human chorionic gonadotropin (Ovitrelle, Merck Serono) (dual trigger) or 250 mcg recombinant human chorionic gonadotropin (Ovitrelle, Merck Serono) (hcg-only trigger). Oocytes are retrieved under transvaginal ultrasound guidance 35–36 h after oocyte maturation triggering. Fertilization was carried out by ICSI. Fertilization was confirmed by observing oocytes with 2 pronuclei (2PN) within 16–18 h following ICSI.

Calcium ionophore (A23187) agent was used for AOA in some patients due to clinicians' preference. In cases that underwent AOA, mature oocytes were exposed to 5 μmol/l A23187 (GM508 CultActive, Gynemed GmbH&Co, Germany) for 15 min following ICSI. Then washed in fresh IVF medium without A23187 and transferred into a drop of IVF medium under mineral oil in disk for incubation. Single-step culture media (global® Collect® HEPES-buffered medium, CooperSurgical Fertility Solutions) are used for culturing of embryos. Quality of blastocysts was assessed through the grading system proposed by the Society for Assisted Reproductive Technology (SART).[8] In accordance with the SART grading system, Grade 1 embryos are referred as good quality embryos, Grade 2 embryos are referred as fair quality embryos, and Grade 3 embryos are referred as poor quality embryos in this study. Soft embryo transfer catheters with guidance of abdominal ultrasonography were used for embryo transfer procedure. A maximum of two embryos was transferred in each cycle. Luteal phase support with intravaginal progesterone is initiated in every patient with either 200 mg Lutinus (Lutinus vaginal tablets, Ferring Pharmaceuticals) twice a day or with 200 mg Progestan 3 times a day (Progestan Soft Capsules, Koçak Farma Pharmaceutical and Chemical Industry Co.) and continued through 8th–10th gestational weeks.

Outcome parameters were defined in line with the International Glossary on Infertility and Fertility Care, 2017.[9] Primary outcome of this study was determined as live birth delivery rates. Secondary outcomes were clinical pregnancy rates, implantation rates, infant birthweight, and gestational ages at delivery.

Statistical analysis was conducted by IBM SPSS 23 (Evaluation version). Descriptive statistics were expressed as mean±standard

Table 1: Comparison of baseline characteristics of women with and without artificial oocyte activation with calcium ionophore

	Calcium ionophore group	Control group	p
Number of patients	185	162	
Age (years)	35.04±5.49	34.17±5.19	0.067
BMI	24.89±2.97	25.18±2.80	0.329
Etiology of infertility			
Anovulation	58 (31.4%)	41 (25.3%)	0.514
Tubal factor	43 (23.2%)	41 (25.3%)	
Endometriosis	22 (11.9%)	19 (11.7%)	
Combined	25 (13.5%)	18 (11.1%)	
Unexplained	37 (20%)	43 (26.5%)	
Number of previous IVF attempts	2.24±1.67	2.22.±1.61	0.953

BMI: Body mass index; IVF: *In vitro* fertilization.

deviations for normally distributed data and as median (min–max) for non-normally distributed data. Categorical variables were expressed as numbers and percentages (%). Significance of differences between means was assessed with Student's t-test and significance of differences between medians was assessed by Mann–Whitney U-test. Categorical variables were assessed with Pearson's Chi-squared test or Fisher's exact test. P<0.05 is considered as statistically significant.

RESULTS

A total of 347 women with a history of previous IVF failure due to embryo developmental arrest were included in this study. One hundred and eighty-five of them were found to undergo AOA with calcium ionophore and 162 of them that treated without AOA were assigned in the control group.

Mean age of calcium ionophore and control groups was 35.04±5.49 and 34.17±5.19 years, respectively. Mean body mass index (BMI) of calcium ionophore group was 24.89±2.97 and 25.18±2.80 for controls. Age and BMI of groups were similar. No significant differences were found concerning distribution of infertility etiologies among groups. Causes of infertility were listed as anovulation, tubal factor, unexplained, endometriosis, and combined in a decreasing prevalence within the study population. Among the etiologies of infertility, "combined" was referred for the patients who have more than 1 of the aforementioned infertility causes.

Mean previously failed IVF attempts due to embryo developmental arrest in calcium ionophore group and controls were 2.24±1.67 and 2.22±1.61, respectively. No significant differences were found in number of previously failed IVF attempts. Characteristics of the study population and controls are given in Table 1.

Mean number of retrieved oocytes, oocyte maturation rates, number of M2 oocytes, and number of 2PN embryos were not significantly different within two groups. Significantly higher rates of good quality embryos were observed to be obtained in women treated with AOA with calcium ionophore (65.3% vs. 31.1%; p<0.001). A total of 170 embryo transfer procedures were applied in the calcium ionophore group and 132 in the control group. Embryo transfer could not be prosecuted for 15 women in the calcium ionophore group and 30 women in the control group due to embryonic developmental arrest and fertilization failure. Significantly higher rates of day 5 embryo transfer were applied in calcium ionophore group in comparison to controls. Implantation rate in the control group was significantly lower in comparison to the calcium ionophore group (0.22±0.39 vs. 0.33±0.41; p=0.003).

Significantly increased clinical pregnancy rates and live birth delivery rates were found in patient with calcium ionophore administration comparing to controls (43.2% vs. 25.3% p<0.001 and 40.5% vs. 21%; p<0.001, respectively). Rate of multiple pregnancy was also found higher in the calcium ionophore group (5.4% vs. 1.9%; p=0.01). Mean infant birth weights were similar among groups. Mean gestational ages at birth were higher than 37 weeks in both groups. There was a slight tendency of delivering at earlier weeks in calcium ionophore group that reach statistical significance. Comparison of cycle outcomes of groups is summarized in Table 2.

DISCUSSION

Results of this study demonstrated that AOA with calcium ionophore increases embryo quality, clinical pregnancy rates, and live birth delivery rates in women with a history of previous IVF failures due to embryo developmental arrest.

AOA causes a prolonged increase in intracellular calcium concentrations. This method is used in an attempt to countervail the supposed deficiency in physiological calcium oscillations generated by increased inositol triphosphate (IP3) concentrations.[10] AOA could be carried out by utilizing mechanical or electrical methods or using chemical agents such as A23187, ionomycin, puromycin, and 6-dimethylaminopurine.[11] Contemporarily, A23187 and ionomycin are most widely used agents in procedures pertaining AOA in human oocytes.[12] AOA is primarily used for cases with male factor infertility and to overcome fertilization failures in assisted reproduction.[13]

Table 2: Comparison of cycle outcomes of women with and without artificial oocyte activation with calcium ionophore

	Calcium ionophore group	Control group	p
Number of patients	185	162	
Required gonadotropin doses (IU)	3402.70±820.83	3296.30±1597.69	0.060
Days of stimulation	9.54±1.64	9.32±1.48	0.290
Peak estradiol level (pg/ml)	1517.85±757.651	1618.20±690.317	0.026
Peak progesterone level (ng/ml)	0.69±0.34	0.77±0.39	0.141
Endometrial thickness (mm)	10.05±1.89	10.20±1.65	0.254
Number of retrieved oocytes	9.51±4.21	9.97±4.35	0.314
Maturation rate (M2 oocytes/retrieved oocytes)	0.81±0.23	0.76±0.15	0.090
Number of M2 oocytes	7.22±2.77	7.38±2.98	0.974
Number of 2PN embryos	4.60±2.59	4.22±2.73	0.211
Quality of obtained embryos			
Good	111 (65.3%)	41 (31.1%)	**<0.001**
Fair	46 (27.1%)	74 (56.1%)	
Poor	13 (7.6%)	17 (12.9%)	
Number of frozen embryos	1.33±0.48	1.22.±0.43	0.382
Number of transferred embryos	1.38±0.64	1.41±0.65	0.412
Day of embryo transfer			
Day 3	67 (39.4%)	99 (75%)	**<0.001**
Day 5	97 (57.1%)	25 (18.9%)	
Day 6	6 (3.5%)	8(6.1%)	
Fertilization rate per cycle	0.63±0.21	0.57±0.28	0.174
Implantation rate per cycle	0.33±0.41	0.22±0.39	**0.003**
Clinical pregnancy rate	80/165 (43.2%)	41/162 (25.3%)	**<0.001**
Live birth delivery rate (n)	75/185 (40.5%)	34/162 (21%)	**<0.001**
Miscarriage	15 (8.1%)	10 (6.2%)	0.905
Multiple pregnancies	10 (5.4%)	3 (1.9%)	**0.010**
Gestational weeks at birth	37.38±2.95	38.28±1.51	**0.043**
Infant birth weight	3099.12±634.78	3164.20±500.08	0.549

PN: Pronuclei. Bold represent the p values <0.05 are considered significant.

However, animal studies have shown that artificially induced calcium oscillations in oocytes have extensive effects on successive embryo development, embryo quality, and implantation rates.[14–16] Besides, these intracellular calcium oscillations found to be associated with alterations in genomic expressions of embryos.[14]

To date, few studies investigated the effects of AOA with calcium ionophore on human embryo development and quality.[17–19] Darwish et al.[17] published a preliminary study conducted on four patients with previous embryonic arrest at 2PN stage. They found increased cleavage rates and increased number of clinical pregnancies following AOA with A23187. Although their sample size was limited and regression to the mean bias should be kept in mind as the study lacks a control group and compares pre- and post-AOA outcomes of same patients, they provide some early evidence for the issue. Ebner et al.[18] conducted a prospective study on 57 patients with a history of complete embryonic developmental arrest in the previous cycles. They have demonstrated that clinical pregnancy rates, implantation rates, blastulation rates, and live birth rates are significantly higher in calcium ionophore cycles. In addition to this, they found higher rates of good quality embryos in women treated with AOA. Yin et al.[19] conducted a study on 140 women with embryo development problems by administering AOA with ionomycin. Contrary to the previous findings, they have reported similar embryonic development, clinical pregnancy rates, and live birth rates among the AOA group and control group.

Parallel with the findings of Ebner et al.,[18] we found significantly higher clinical pregnancy rates, live birth delivery rates, and implantation rates in patients treated with A23187 in comparison to controls. Embryo quality was also significantly improved.

In our study, multiple pregnancy rate in the calcium ionophore group was significantly higher in comparison to controls. A previous study conducted by Shebl et al.[20] who also reported higher multiple pregnancy rates in women with calcium ionophore oocyte activation. They concluded that higher rates of blastocyst transfer in the calcium ionophore group might be the cause of higher multiple pregnancy rates. In our study, although the mean number of transferred embryos was similar among groups, rate of day 5 blastocyst transfers as well as the quality of embryos was significantly improved in the calcium ionophore group which could be the factors lying behind higher multiple pregnancy rates in this group.

Previously, physiological frequency of calcium oscillations was shown to be associated with embryo implantation potential.[16] Calcium signaling in early post-fertilization phase, pronuclear phase, and cleavage phases is known to exhibit distinct unique characteristics.[5] Calcium ionophore agents create a single prolonged intracellular increase in calcium concentrations. Besides lacking a close resemblance to physiologic calcium oscillations, widely used agents such as A23187 and ionomycin have different calcium transport properties.[21,22] Discrepancies in features of A23187 and ionomycin might be the cause of contrasting results of the studies in the literature.

At present, studies evaluating the effects of AOA with calcium ionophore on neonatal outcomes did not found any association between adverse obstetric outcomes and AOA.[13,23–25] In our study, mean gestational weeks of AOA group and controls were both found above 37 weeks. Although a slight tendency to deliver in earlier weeks in the calcium ionophore group was noted comparing to controls, clinical impact of this difference is dubious. Mean infant birth weights in both groups were also found similar.

At present, AOA with calcium ionophore is mainly utilized in patients with fertilization failure in daily practice. There are few studies in the literature evaluating the effects of AOA in women with previous IVF failures due to embryonic developmental arrest, with limited sample sizes. Relatively large study population size is the major strength of this study while retrospective design is the main limitation which curbs this study to conclude this topic.

CONCLUSION

AOA with A23187 seems to improve progression to blastocyst rates, embryo quality, clinical pregnancy rates, and live birth delivery rates in women with a previous history of complete embryo development arrest. Further prospective studies are required to effectively clarify this issue.

Statement

Ethics Committee Approval: The Üsküdar University Non-Interventional Research Ethics Committee granted approval for this study (date: 30.04.2021, number: 61351342/April 2021-85).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – FT; Design – FT, AK; Supervision – AK; Resource – AK; Materials – FT; Data Collection and/or Processing – FT; Analysis and/or Interpretation – AK; Literature Search – FT, AK; Writing – AK; Critical Reviews – FT.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Glujovsky D, Farquhar C, Quinteiro Retamar AM, Alvarez Sedo CR, Blake D. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. Cochrane Database Syst Rev 2016;CD002118.
2. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: Proceedings of an expert meeting. Hum Reprod 2011;26:1270–83.
3. Sfakianoudis K, Maziotis E, Karantzali E, Kokkini G, Grigoriadis S, Pantou A, et al. Molecular drivers of developmental arrest in the human preimplantation embryo: A systematic review and critical analysis leading to mapping future research. Int J Mol Sci 2021;22:8353.
4. ESHRE Special Interest Group of Embryology; Alpha Scientists in Reproductive Medicine. The Vienna consensus: Report of an expert meeting on the development of art laboratory performance indicators. Hum Reprod Open 2017;2017:hox011.
5. Tesarik J. Calcium signaling in human preimplantation development: A review. J Assist Reprod Genet 1999;16:216–20.
6. Wong R, Hadjiyanni I, Wei HC, Polevoy G, McBride R, Sem KP, et al. PIP2 hydrolysis and calcium release are required for cytokinesis in Drosophila spermatocytes. Curr Biol 2005;15:1401–6.
7. Sousa M, Barros A, Tesarik J. Developmental changes in calcium dynamics, protein kinase C distribution and endoplasmic reticulum organization in human preimplantation embryos. Mol Hum Reprod 1996;2:967–77.
8. Racowsky C, Vernon M, Mayer J, Ball GD, Behr B, Pomeroy KO, et al. Standardization of grading embryo morphology. J Assist Reprod Genet 2010;27:437–9.
9. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, et al. The international glossary on infertility and fertility care, 2017. Fertil Steril 2017;108:393–406.
10. Kang HJ, Lee SH, Park YS, Lim CK, Ko DS, Yang KM, et al. Artificial oocyte activation in intracytoplasmic sperm injection cycles using testicular sperm in human *in vitro* fertilization. Clin Exp Reprod Med 2015;42:45–50.
11. Heindryckx B, De Sutter P, Gerris J, Dhont M, Van der Elst J. Embryo development after successful somatic cell nuclear transfer to *in vitro* matured human germinal vesicle oocytes. Hum Reprod 2007;22:1982–90.
12. Kashir J, Heindryckx B, Jones C, De Sutter P, Parrington J, Coward K. Oocyte activation, phospholipase C zeta and human infertility. Hum Reprod Update 2010;16:690–703.
13. Shan Y, Zhao H, Zhao D, Wang J, Cui Y, Bao H. Assisted oocyte activation with calcium ionophore improves pregnancy outcomes and offspring safety in infertile patients: A systematic review and meta-analysis. Front Physiol 2022;12:751905.
14. Ozil JP, Banrezes B, Tóth S, Pan H, Schultz RM. Ca2+ oscillatory pattern in fertilized mouse eggs affects gene expression and development to term. Dev Biol 2006;300:534–44.
15. Bos-Mikich A, Whittingham DG, Jones KT. Meiotic and mitotic Ca2+ oscillations affect cell composition in resulting blastocysts. Dev Biol 1997;182:172–9.

16. Knott JG, Kurokawa M, Fissore RA, Schultz RM, Williams CJ. Transgenic RNA interference reveals role for mouse sperm phospholipase Czeta in triggering Ca2+ oscillations during fertilization. Biol Reprod 2005;72:992–6.
17. 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. Reprod Biomed Online 2015;31:799–804.
18. Ebner T, Oppelt P, Wöber M, Staples P, Mayer RB, Sonnleitner U, et al. Treatment with Ca2+ ionophore improves embryo development and outcome in cases with previous developmental problems: A prospective multicenter study. Hum Reprod 2015;30:97–102.
19. Yin M, Li M, Li W, Wu L, Yan Z, Zhao J, et al. Efficacy of artificial oocyte activation in patients with embryo developmental problems: A sibling oocyte control study. Arch Gynecol Obstet 2022;305:1225–31.
20. Shebl O, Reiter E, Enengl S, Allerstorfer C, Schappacher-Tilp G, Trautner PS, et al. Double ionophore application in cases with previous failed/low fertilization or poor embryo development. Reprod Biomed Online 2022;44:829–37.
21. Erdahl WL, Chapman CJ, Taylor RW, Pfeiffer DR. Ca2+ transport properties of ionophores A23187, ionomycin, and 4-BrA23187 in a well defined model system. Biophys J 1994;66:1678–93.
22. Wang E, Taylor RW, Pfeiffer DR. Mechanism and specificity of lanthanide series cation transport by ionophores A23187, 4-BrA23187, and ionomycin. Biophys J 1998;75:1244–54.
23. Mateizel I, Verheyen G, Van de Velde H, Tournaye H, Belva F. Obstetric and neonatal outcome following ICSI with assisted oocyte activation by calcium ionophore treatment. J Assist Reprod Genet 2018;35:1005–10.
24. Miller N, Biron-Shental T, Sukenik-Halevy R, Klement AH, Sharony R, Berkovitz A. Oocyte activation by calcium ionophore and congenital birth defects: A retrospective cohort study. Fertil Steril 2016;106:590–6.e2.
25. Bonte D, Ferrer-Buitrago M, Dhaenens L, Popovic M, Thys V, De Croo I, 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. Fertil Steril 2019;112:266–74.