Akpınar *et al. Middle East Fertility Society Journal* (2026) 31:21
https://doi.org/10.1186/s43043-026-00311-7

Middle East Fertility Society Journal

RESEARCH Open Access

Artificial oocyte activation in ICSI cycles in severe male infertility cases: is calcium ionophore or piezoelectric better?

Ayşe Altun Akpınar[1], Burçin Karamustafaoğlu Balcı[1*], Çiğdem Çelik[1], Büşra Duymaz Edgü[1] and Cemil Akgül[1]

Abstract

Objective To compare the effectiveness of calcium ionophore–induced artificial oocyte activation (AOA) and piezoelectric stimulation in improving fertilization, embryo quality, and pregnancy outcomes in ICSI cycles of severe male factor infertility.

Methods A prospective controlled study was conducted at the Istanbul University IVF Center between December 2023 and June 2025. Data collection and follow-up for all pregnancy outcomes, including live birth, were completed before July 2025. The study included 150 couples with severe male factor infertility divided into three groups: (i) Control (conventional ICSI, $n=50$), (ii) ICSI + Calcium ionophore ($n=50$), and (iii) ICSI + Piezoelectric ($n=50$). Severe male factor infertility was defined according to WHO (2021) criteria. Oocytes were fertilized and cultured under standardized conditions. Embryo morphology was assessed on Day 3 according to the Istanbul Consensus (2011) criteria and on Day 5 using the Gardner and Schoolcraft grading system. Statistical comparisons were performed using ANOVA, Kruskal–Wallis, and chi-square tests, with $p<0.05$ considered significant. The primary outcome measure was the fertilization rate, while embryo quality and clinical outcomes were evaluated as secondary outcomes.

Results Fertilization rates were significantly higher in the ICSI+Piezoelectric group compared with both the control and ICSI+Calcıum ionophore groups ($p<0.0001$). The proportion of good-quality (Grade I) embryos was also greater in the ICSI+Piezoelectric group (38.2%) than in the control (28.3%) and ICSI+Calcıum ionophore (29.5%) groups ($p<0.05$). No statistically significant differences were observed in pregnancy outcomes, including implantation, clinical pregnancy, ongoing pregnancy, or live birth rates; however, a non-significant trend toward higher ongoing pregnancy rates was noted in the ICSI+Calcıum ionophore group.

Conclusions Piezoelectric activation was associated with higher fertilization rates and improved early embryo morphology; however, these improvements did not translate into statistically significant differences in clinical pregnancy or live birth outcomes.

Keywords Piezoelectric activation, Intracytoplasmic sperm injection (ICSI), Calcium ionophore, Oocyte activation, Embryo quality, Severe male infertility, Assisted reproduction

*Correspondence:
Burçin Karamustafaoğlu Balcı
burcinkaramustafaoglu@yahoo.com
[1]Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Faculty of Medicine, Istanbul University, Capa, Fatih, Istanbul, Turkey

© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

Introduction

Male factor infertility remains a significant contributor to subfertility globally, particularly in scenarios involving severe sperm abnormalities such as extreme oligospermia, asthenozoospermia, or teratozoospermia. Despite the widespread use of intracytoplasmic sperm injection (ICSI), fertilization failure (often stemming from oocyte activation deficiency (OAD)) continues to impede successful outcomes [1, 2].

For many years, the paternal contribution to early embryogenesis was largely regarded as a simple carrier of the paternal genome. However, recent research underscores that sperm deliver far more than DNA: they convey centrosomes, activation factors, RNAs—including mRNAs and small non-coding RNAs—and epigenetic information such as histone modifications and DNA methylation patterns that can profoundly influence zygotic and preimplantation development [3, 4]. These paternal factors are now recognized as critical determinants of embryo quality, affecting pronuclear formation, cleavage dynamics, and subsequent developmental competence [4, 5]. Studies have demonstrated that deficiencies in sperm-derived components can manifest as early as the first cell cycle—resulting in abnormal pronuclear morphology, delayed cleavage, or increased fragmentation—suggesting that paternal effects commence at the very onset of embryogenesis [6, 7].

Oocyte activation deficiency remains a critical factor underlying fertilization failure rates, which can reach up to 5% in some series [1]. Artificial oocyte activation (AOA) has been adopted as a salvage technique when natural activation fails. AOA methods include chemical approaches using calcium ionophores such as ionomycin or A23187, which generate an artificial surge of Ca^{2}, and physical methods like electrical or piezoelectric stimulation which transiently perturb the oolemma to facilitate Ca^{2+} influx [8, 9].

Meta-analytic evidence indicates that AOA significantly enhances fertilization and live birth rates in patients with previous fertilization failure: fertilization odds approximately triple and live birth odds rise more than ten-fold, whereas no significant benefit is observed in patients without prior activation failure [1, 10]. In a subset of patients with confirmed PLCζ deficiency, ICSI combined with AOA dramatically improved outcomes: live birth rates of 66.3% compared to merely 4.6% in non-AOA controls, with significantly higher cumulative clinical pregnancy and live birth rates [11]. Despite these encouraging clinical results, the artificial Ca^{2+} elevation introduced by ionophores—typically a single, non-oscillatory spike—may not faithfully recapitulate physiological Ca^{2+} oscillations [12]. This raises concerns about the correct activation of downstream signalling cascades including CaMKII, MPF decay, DAG/PKC pathways, mitochondrial stability, and epigenetic regulation [13].

Piezoelectric activation offers a potential alternative, hypothesised to better mimic natural Ca^{2+} dynamics by mechanically inducing transient membrane perturbations. A 2025 sibling-oocyte study reported comparable fertilization and blastulation rates between piezoelectric and calcium ionophore protocols, suggesting potential equivalency in early developmental outcomes [14]. However, rigorous, controlled clinical studies directly comparing these AOA modalities in the context of severe male factor infertility are scarce.

In light of the mechanistic importance of PLCζ-mediated activation, the theoretical limitations of current chemical AOA, and preliminary evidence supporting piezoelectric methods, we designed a comparative clinical study. This study aimed to evaluate the efficacy of calcium ionophore versus piezoelectric AOA in ICSI cycles involving severe sperm abnormalities, such as extreme oligospermia, asthenozoospermia, or teratozoospermia, compared to control cycles without male factor infertility.

Materials and methods

Study design and patient selection

This prospective controlled study was conducted at the IVF Center of Istanbul University, Istanbul Faculty of Medicine between December 2023 and June 2025. All data collection and follow-up, including live birth outcomes, were completed before July 2025. Ethical approval was obtained from the Institutional Review Board (approval number:21.12.2023-E-29624016-050.99-99-2310074), and written informed consent was obtained from all participants.

The primary outcome measure of the study was the fertilization rate, defined as the proportion of normally fertilized oocytes (2PN) per injected metaphase II oocyte. Secondary outcomes included embryo quality parameters and clinical outcomes, such as implantation rate, clinical pregnancy rate, and ongoing pregnancy rate.

The study included three groups:

- Control group (conventional ICSI): 50 patients with severe male factor infertility who underwent ICSI without artificial oocyte activation.
- ICSI+ Calcium ionophore group: 50 patients with severe male factor infertility who underwent ICSI followed by calcium ionophore–induced artificial oocyte activation.
- ICSI+Piezoelectric group: 50 patients with severe male factor infertility who underwent ICSI followed by piezoelectric artificial oocyte activation.

Severe male factor infertility was defined as sperm concentration < 5 million/mL, progressive motility < 10%,

or morphology < 1% according to WHO 2021 criteria. The study groups were formed sequentially rather than randomized due to the phased implementation of the piezoelectric system in our IVF unit. At the time of study initiation, conventional ICSI and calcium ionophore–assisted ICSI were routinely available, whereas piezoelectric activation became available later during the study period. All patients were included prospectively according to predefined inclusion criteria and treated consecutively within each group.

Ovarian stimulation and oocyte collection.

Controlled ovarian stimulation was performed using standard antagonist protocols. Oocytes were retrieved 36 h after hCG trigger and denuded to assess nuclear maturity. Only metaphase II (MII) oocytes were included in the study.

Intracytoplasmic sperm injection and artificial oocyte activation.

1. Control group
    - ICSI was performed without artificial activation.
2. ICSI+Calcıum ionophore group
    - After ICSI, oocytes were exposed to Ca^{2+} ionophore (Gynemed, Germany; distributed by Biltek, Turkey).
    - Immediately after ICSI, The oocytes were incubated in ionophore-containing medium for 20 minutes at 37 °C in 5% CO2 and then transferred back to fresh culture medium.
3. ICSI+Piezoelectric group
    - After ICSI, oocytes were incubated for 30 minutes before artificial activation.
    - Oocytes were then exposed to piezo stimulation using the Zeus piezoelectric system (Zeus Co., Japan), according to the manufacturer's protocol.

Fertilization assessment

Fertilization was evaluated 16–18 h post-ICSI. Normal fertilization was defined as the presence of two pronuclei (2PN) and two polar bodies. Oocytes without pronuclei or with abnormal fertilization were excluded from embryo development assessment. Fertilization rate was calculated for each patient as the number of fertilized oocytes divided by the total number of injected oocytes, and statistical analyses were performed using patient-level data.

Embryo culture and grading

Embryo development was assessed at both the cleavage stage (day 2–3) and the blastocyst stage (day 5–6), depending on the individualized clinical transfer strategy. Cleavage-stage embryos were graded according to standard morphological criteria, including blastomere number, symmetry, and fragmentation, while blastocysts were evaluated based on expansion, inner cell mass, and trophectoderm quality.

To enable a unified comparison across patients with different transfer timings, embryos were subsequently classified into three overall developmental quality categories (good, moderate, poor) based on their stage-appropriate morphological grade, irrespective of the day of development. This day-independent classification was used for statistical analysis.

Day 2/3 embryos were evaluated for cleavage, blastomere number, symmetry, and fragmentation according to the Istanbul Consensus (2011).

Embryo grading at the on Day 3

– Blastomere Number and Symmetry
    - A high-quality Day 3 embryo typically contains 6–8 blastomeres (cells).
    - The blastomeres should be uniform in size and shape, as irregularities may indicate abnormal division.

– Fragmentation Rate
    - Fragmentation refers to the presence of small, anucleate cytoplasmic fragments within the embryo.
    - The grading system is generally as follows:
        - Grade 1 (0% fragmentation): No fragments, evenly sized blastomeres-Excellent quality.
        - Grade 2 (10% fragmentation): Few small fragments -Good quality.
        - Grade 3 (10–25% fragmentation): Moderate fragments, slightly uneven blastomeres-Fair quality.
        - Grade 4 (25% fragmentation): Significant fragmentation, irregular blastomeres-Poor quality.

– Multinucleation
    - Ideally, each blastomere should contain a single nucleus.

- The presence of multinucleated cells is associated with lower implantation potential and increased chromosomal abnormalities.

Day 5/6 blastocysts were graded using the Gardner and Schoolcraft system, based on expansion, inner cell mass (ICM), and trophectoderm (TE) morphology.

Embryo grading at the on Day 5

asasasasas

– Number (1-6): Blastocyst expansion and hatching stage

1. Early blastocyst
2. Blastocoel fills more than 50% of the embryo
3. Full blastocyst (blastocoel completely expanded)
4. Expanded blastocyst (zona pellucida starts thinning)
5. Hatching blastocyst (partially hatched)
6. Fully hatched blastocyst

– First Letter (1, 2, 3): Inner Cell Mass (ICM) Quality (Determines the cells that will form the baby)

1. Many tightly packed cells, well-organized
2. Fewer cells, loosely packed
3. Very few cells, irregular structure

– Second Letter (1, 2, 3): Trophectoderm Quality (Determines the cells that will form the placenta)

1. Many cells, forming a uniform layer
2. Moderate number of cells, slightly irregular
3. Few cells, highly irregular

(Gardner & Schoolcraft. In vitro culture of human blastocysts.)

Embryo transfer

Embryo transfer was performed on Day 2, Day 3, or Day 5 under ultrasound guidance by the same infertility and reproductive endocrinology specialist experienced in IVF procedures. The day of embryo transfer (cleavage-stage or blastocyst-stage) was determined according to routine clinical practice and individual embryo development.

All embryo transfers were performed as fresh embryo transfer cycles. The number of embryos transferred (single or double embryo transfer) was determined based on patient age, embryo availability, and national regulations. Both first and repeated embryo transfer cycles were included in the study.

All patients received luteal support with vaginal 200 mg micronised progesterone 3 times/dayand daily 50 mg intramuscular progesterone. Surplus embryos were cryopreserved for subsequent cycles. In cases using the PPOS protocol, with suboptimal endometrial appearance, or at risk of ovarian hyperstimulation syndrome (OHSS), all embryos were cryopreserved.

These embryo transfer characteristics were comparable across the study.

Outcome measures

- Fertilization rate (2PN / injected MII oocytes).
- Cleavage rate (≥ 2-cell embryos / normally fertilized oocytes).
- Blastocyst formation rate (blastocysts / cleaved embryos).
- High-quality embryo rate (day 3 and day 5).
- Clinical outcomes; implantation rate, clinical pregnancy rate and ongoing pregnancy rate.

Statistical analysis

The normality of the data distribution was assessed using the Shapiro–Wilk test. For variables showing a normal distribution, comparisons among groups were performed using one-way ANOVA, and if significant, post-hoc pairwise comparisons were conducted with the Tukey–Bonferroni test.For variables not following a normal distribution, non-parametric tests were applied, and descriptive statistics were presented as median (IQR: Q1–Q3). Group comparisons for non-normally distributed variables were performed using the Kruskal–Wallis test, and in case of significant differences, pairwise comparisons were conducted with the Dunn–Bonferroni post-hoc test.Categorical variables were compared among groups using the Pearson chi-square test. A p-value < 0.05 was considered statistically significant in all analyses.

Results

The demographic and laboratory characteristics of the groups demonstrated some significant differences (Table 1). Female age showed a statistically significant difference among the three groups. The mean age of the control group was 31.9 ± 4.2; ICSI+Calcıum ionophore group 34.4 ± 4.6 and ICSI+Piezoelectric group 32.5 ± 3.9 (p:0.0186). The ICSI+Calcıum ionophore group was 2 years older than the control and ICSI+Piezoelectric group. Because female age differed between groups, a multivariable linear regression analysis was performed to adjust for potential confounding. Female age was not independently associated with fertilization rate ($\beta = 0.0006$, $p = 0.91$). Likewise, ovarian reserve markers including AMH ($p = 0.91$), FSH ($p = 0.51$), and antral follicle count ($p = 0.99$) were not significant predictors. After adjustment for these variables, the ICSI+Piezoelectric

Table 1 Comparison of baseline demographic characteristics, oocyte yield, and fertilization outcomes among the three study groups (Control, ICSI + Calcium ionophore, and ICSI + Piezoelectric). The table presents female and male age, the total number of retrieved oocytes, and the number of metaphase II (MII) oocytes. In addition, fertilization rates are shown both as mean ± SD and as median values with interquartile ranges (IQR). Pairwise comparisons highlight significant differences between groups

	Control Group		ICSI+Calcium ionophore Group		ICSI+Piezoelectric Group		*p* value	Pairwise comparisons *p*-value
	n	Mean ± S.D.	*n*	Mean ± S.D.	*n*	Mean ± S.D.		
Female Age	50	31.9 ± 4.2	50	34.4 ± 4.6	50	32.5 ± 3.9	0.0186*	Control-Piezo: $p < 0.05$. Calcıum-Piezo: $p < 0.05$.
Male Age	50	35.8 ± 3.6	50	37.3 ± 5.1	50	34.9 ± 4.1	0.055	
Total oocyte count	50	9.06 ± 4.01	50	6.32 ± 3.41	50	8.26 ± 4.59	0.0019*	Control-Calcıum: $p < 0.05$.
MII oocyte count	50	7.22 ± 3.45	50	4.74 ± 3.30	50	6.28 ± 3.87	0.0017*	Control-Calsiyum: $p < 0.05$. Calcıum-Piezo: $p < 0.05$.
Fertilization rate (%±S.D)	50	57.3 ± 31.3	50	55.6 ± 31	50	84 ± 22.3	$p < 0.0001$*	Control-Piezo: $p < 0.05$. Calcıum-Piezo: $p < 0.05$.
Fertilization rate Median (IQR)	50	63 (36.8–80)	50	55.5 (33–75)	50	100 (70–100)		

Female age, male age: ANOVA test was used, for pairwise comparisons, the Tukey post-hoc test with Bonferroni correction was applied. Total oocyte count, MII oocyte count, Fertilization count: Kruskal Wallis was used, pairwise comparisons were performed using the Dunn post-hoc test with Bonferroni correction

* Significant at $p < 0,05$

Table 2 Multivariable linear regression analysis was performed to evaluate the independent effects of female age, ovarian reserve parameters (FSH, AMH, AFC), and treatment group on fertilization rate

Variable	β (Beta)	*p* value
Female age (years)	0.0006	0.91
FSH	−0.0045	0.51
AMH	−0.0009	0.91
Antral follicle count (AFC)	−0.0001	0.99
ICSI+ Piezoelectric group (vs. conventional ICSI)	0.285	< 0.00001
ICSI+Calcium ionophore group (vs. conventional ICSI)	0.11	0.70

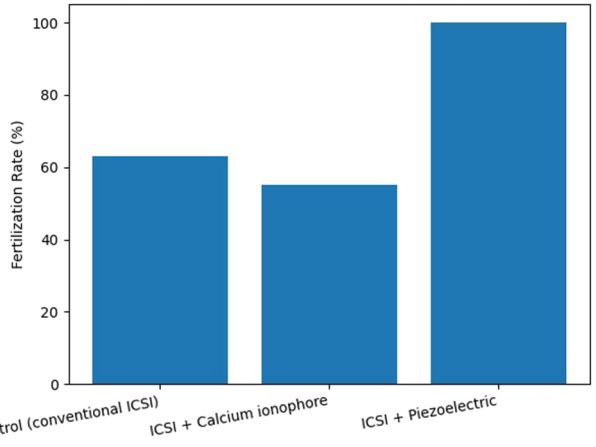


Fig. 1 Fertilization rates among study groups

group remained a strong independent predictor of higher fertilization rate (β = 0.285, $p < 0.00001$), whereas the ICSI+Calcıum ionophore group did not differ from control ($p = 0.70$), (Table 2). Male age did not differ significantly among the groups ($p = 0.055$), indicating a comparable male demographic profile.

In what concerns ovarian response of the groups;

- Control group: 50 patients severe male infertility factor, with a mean of 7.22 ± 3.45 MII oocytes per patient (total number of MII oocytes: 361).
- ICSI+Calcıum ionophore group: 50 patients with severe male factor infertility, with a mean of 4.74 ± 3.30 MII oocytes per patient (total number of MII oocytes: 237).
- ICSI+Piezoelectric group: 50 patients with severe male factor infertility, with a mean of 6.28 ± 3.87 MII oocytes per patient (total number of MII oocytes: 314).

The total oocyte count was significantly reduced in the ICSI+Calcıum ionophore group compared to the control ($p = 0.0019$). Similarly, the MII oocyte count was also lower in the ICSI+Calcıum ionophore group when compared with both the control and ICSI+Piezoelectric groups ($p = 0.0017$). These results can be explained with the age difference between the ICSI+Calcıum ionophore group and the two other groups (Table 1).

The most striking difference was observed in fertilization rates, which were significantly higher in the ICSI+Piezoelectric group compared to both the control and ICSI+Calcıum ionophore groups ($p < 0.0001$). Pairwise comparisons confirmed that piezoelectric activation provided a superior fertilization outcome ($p < 0.05$). Median (IQR) fertilization rates further supported this observation, with values of 63 (36.8–80) in the control group, 55.5 (33–75) in the ICSI+Calcıum ionophore, and 100 (70–100) in the ICSI+Piezoelectric group (Table 1). Fertilization rates across the three study groups are illustrated in Fig. 1, demonstrating a significantly higher

Table 3 Comparison of embryo quality among the three study groups (Control, ICSI + Calcium ionophore, and ICSI + Piezoelectric). The table summarizes the distribution of embryos according to morphological grading: good-quality embryos (Grade I), average-quality embryos (Grade II), poor-quality embryos (Grade III), and unfertilized or arrested embryos. Significant differences were observed particularly in the distribution of Grade II, Grade III, and unfertilized/arrested embryos among the groups

	Control Group	ICSI+Calcium ionophore Group	ICSI+Piezoelectric Group	*p* value	Pairwise comparisons (*p* value)
Number of Good-quality embryos (GI)	102/361 (%28.3)	70/237 (%29.5)	120/314 (%38.2)	0.014*	Control–Calcium: $p = 0.81$ Control–Piezo: $p = 0.0077$* Calcium–Piezo: $p = 0.042$*
Number of Average quality embryos (GII)	78/361 (%21.6)	49/237 (%20.7)	99/314 (%31.5)	0.0028*	Control–Calcium: $p = 0.86$ Control–Piezo: $p = 0.0046$* Calcium–Piezo: $p = 0.0060$*
Number of Poor quality embryos (GIII)	20/361 (%5.5)	13/237 (%5.5)	40/314 (%12.7)	$p < 0.001$*	Control–Calcium: $p = 1.0$ Control–Piezo: $p = 0.0017$* Calcium–Piezo: $p = 0.0067$*
Number of unfertilized or arrested embryos	161/361 (%44.6)	105/237 (%44.3)	55/314 (%17.5)	$p < 0.001$*	Control–Calcium: $p = 0.91$ Control–Piezo: $p < 0.0001$* Calcium–Piezo: $p = 0.0001$*

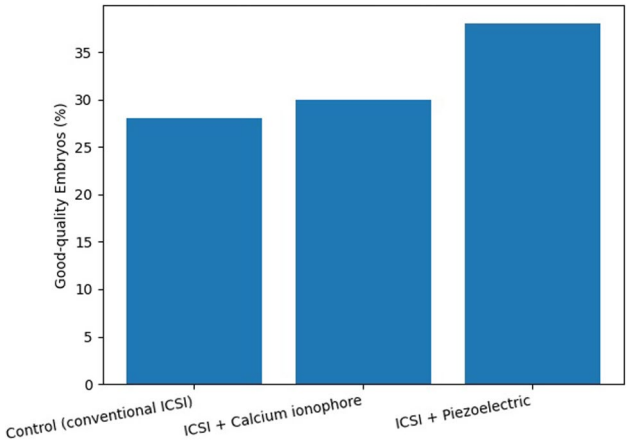


Fig. 2 Proportion of good-quality (Grade I) embryos

fertilization rate in the ICSI+Piezoelectric group compared with both the control and ICSI+Calcıum ionophore groups.

The comparison of embryo quality among the three study groups is summarized in Table 3. A significant difference was observed in the distribution of embryos according to morphological grading ($p < 0.05$). The proportion of good-quality embryos (Grade I) was significantly higher in the ICSI + Piezoelectric group (38.2%) compared with both the control (28.3%, $p = 0.0077$) and ICSI + Calcium ionophore (29.5%, $p = 0.042$) groups. Similarly, the percentage of average-quality embryos (Grade II) was also greater in the ICSI + Piezoelectric group (31.5%) than in the control (21.6%, $p = 0.0046$) and ICSI + Calcium ionophore (20.7%, $p = 0.0060$) groups. In contrast, the incidence of poor-quality embryos (Grade III) was significantly higher in the ICSI + Piezoelectric group (12.7%) compared with both the Control (5.5%, $p = 0.0017$) and ICSI + Calcium ionophore (5.5%, $p = 0.0067$) groups. No significant differences were observed between the ontrol and ICSI + Calcium ionophore groups for any of the embryo quality categories. The rate of unfertilized or developmentally arrested embryos was significantly lower in the ICSI+Piezoelectric group (17.5%) compared to both the control (44.6%) and ICSI+Calcium ionophore (44.3%) groups ($p < 0.001$). There was no significant difference between the control and ICSI+Calcıum ionophore groups ($p = 0.91$), whereas both showed markedly higher rates compared to the ICSI+Piezoelectric group (Control–Piezoelectiric: $p < 0.0001$; Calcium–Piezoelectric: $p = 0.0001$). The distribution of embryo quality grades among the study groups is shown in Fig. 2. The ICSI+Piezoelectric group exhibited a higher proportion of good-quality (Grade I) embryos, as well as a broader distribution of embryo grades, compared with the control and ICSI+Calcıum ionophore groups.

When pregnancy outcomes were compared, no statistically significant differences were observed among the groups. The implantation rates were 30% (9/30) in the control group, 48% (13/27) in the ICSI+Calcıum ionophore group, and 33% (12/36) in the ICSI+Piezoelectric group ($p = 0.32$). Although the ICSI+Calcıum ionophore group showed a numerically higher rate, the difference did not reach statistical significance. Similarly, clinical pregnancy rates were 27% (8/30), 44% (12/27), and 25% (9/36) in the control, ICSI+Calcıum ionophore, and ICSI+Piezoelectric groups, respectively ($p = 0.21$). Again, the ICSI+Calcıum ionophore group demonstrated a non-significant trend toward higher pregnancy and live birth rates, but without statistical significance.

For ongoing pregnancy, the rates were 23% (7/30) in the control, 41% (11/27) in the ICSI+Calcıum ionophore, and 17% (6/36) in the ICSI+Piezoelectric group ($p = 0.12$). This pattern suggests that calcium ionophore activation may be associated with improved pregnancy continuation, though the sample size may not have been sufficient to establish significance, neither causality.

Table 4 Comparison of reproductive outcomes among the three study groups (Control, ICSI + Calcium ionophore, and ICSI + Piezoelectric). The table presents the rates of biochemical pregnancy (β-hCG positivity), clinical pregnancy, ongoing pregnancy, and live birth. Data are shown as the number of events per total cases with corresponding percentages. Although higher rates were observed in the calcium-assisted ICSI group across several outcomes, the differences among groups did not reach statistical significance

Biochemical Pregnancy	Control Group	ICSI+Calcium ionophore Group	ICSI+Piezoelectric Group	*p* value
	9/30 (% 30)	**13/27 (% 48)**	**12/36 (% 33)**	**0.32**
Clinical Pregnancy	8/30 (% 27)	12/27 (% 44)	9/36 (% 25)	0.21
Ongoing Pregnancy	7/30 (% 23)	11/27 (% 41)	6/ 36 (% 17)	0.12
Live Birth	7/30 (% 23)	11/27 (% 41)	6/36 (%17)	0.09

Comparisons among the groups in Table 3 were performed using the Pearson's chi-square test

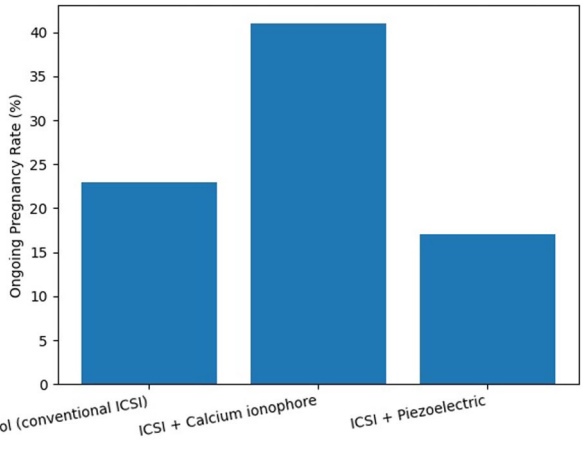


Fig. 3 Ongoing pregnancy rates among study groups

The live birth rates were 23% (7/30) in the control, 41% (11/27) in the ICSI+Calcıum ionophore, and 17% (6/36) in the ICSI+Piezoelectric group ($p = 0.09$). Although not statistically significant, the ICSI+Calcıum ionophore group showed a consistent trend toward higher pregnancy continuation and live birth compared to the other groups (Table 4). Pregnancy outcomes, including implantation, clinical pregnancy, ongoing pregnancy, and live birth rates, are summarized in Fig. 3. No statistically significant differences were observed among the groups, although a non-significant trend toward higher pregnancy outcomes was noted in the ICSI+Calcıum ionophore group.

Discussion

In this study, conventional ICSI, ICSI with calcium ionophore activation, and piezoelectric-assisted ICSI were compared for fertilization outcomes, embryo quality, and pregnancy parameters. The most notable finding was that piezoelectric activation significantly enhanced fertilization rates and increased the number of embryos obtained compared with both conventional ICSI and calcium ionophore treatment. In this study, ICSI+Piezoelectric led to more good- and average-quality embryos, less unfertilized oocytes, less arrest embryos but it was also accompanied by an increase in poor-quality embryos. This broader distribution suggests that piezoelectric activation may rescue oocytes that would otherwise fail to activate, thereby increasing heterogeneity in embryo quality. However, these early laboratory improvements did not consistently translate into higher clinical pregnancy nor live birth rates. Conversely, calcium ionophore activation was associated with numerically higher implantation rate, clinical pregnancy, and ongoing pregnancy rates, although ICSI+Calcıum ionophore group was two years older than the other two groups.

Although piezoelectric activation significantly improved fertilization and early embryological parameters, these advantages did not result in statistically significant improvements in implantation, clinical pregnancy, or live birth rates, underscoring the multifactorial nature of reproductive success.

Recent evidence indicates that impaired sperm DNA integrity and defective chromatin decondensation are associated with reduced PLCζ efficacy, which may explain fertilization failure after ICSI and justify the use of artificial oocyte activation methods such as calcium ionophore or piezoelectric stimulation in severe male infertility cases [15]. Cavarocchi et al. highlighted that genetic and molecular abnormalities observed in severe asthenozoospermia can compromise post-ICSI fertilization outcomes, supporting the implementation of adjunctive clinical strategies such as artificial oocyte activation to improve reproductive success [16]. Advanced imaging approaches have revealed the complex three-dimensional and dynamic nature of human sperm motility, highlighting functional heterogeneity that may not be captured by conventional assessments. Such detailed characterization is particularly relevant in severe male infertility, where impaired sperm function may necessitate adjunctive strategies such as artificial oocyte activation following ICSI [17].

Our results align with earlier reports demonstrating that ICSI+Piezoelectric improves fertilization efficiency and embryo yield. Kimura &Yanagimachi first described the method in mice, showing higher survival and fertilization after membrane penetration [18]. Subsequent clinical studies confirmed superior fertilization and sometimes improved blastocyst formation compared with conventional ICSI [19–21]. In our study, ICSI+Piezoelectric also led to more good- and average-quality embryos, but this was accompanied by an

increase in poor-quality embryos. This broader distribution has been noted previously, suggesting that piezo activation may rescue oocytes that would otherwise fail to activate, thereby increasing heterogeneity in embryo quality. In contrast, calcium ionophore activation failed to improve embryo quality, possibly due to its non-specific and excessive calcium influx, which may disrupt normal oscillation patterns [22, 23], or may be due to the fact that the ICSI+Calcıum ionophore group was two years older than the other groups. One possible explanation may be that piezoelectric activation may reduce mechanical damage to the oolemma while promoting synchronized oocyte activation, resulting in superior embryo morphology [24]. The significantly lower rate of unfertilized or arrested embryos observed in the ICSI+Piezoelectric group suggests that Piezoelectric activation may induce a more physiological pattern of oocyte activation compared to chemical stimulants such as calcium ionophore. Piezo-mediated activation mimics the natural sperm-induced calcium oscillations more closely, which are essential for meiotic resumption and subsequent embryonic development [25, 26].Previous studies have also demonstrated that excessive or artificial calcium influx from ionophore exposure can lead to abnormal cytoplasmic signaling, impaired embryo quality, and higher arrest rates [27, 28]. In contrast, piezoelectric activation has been associated with improved fertilization outcomes and embryo development, particularly in cases of severe male factor infertility [29, 30]. Taken together, our findings are consistent with the literature, supporting that Piezoelectric activation provides a more controlled and biocompatible alternative to chemical activation, enhancing early embryonic competence and reducing the risk of developmental arrest.

Regarding calcium ionophore activation, our findings partially mirror prior meta-analyses showing that ionophore-assisted oocyte activation improves fertilization, cleavage, and implantation rates, particularly in couples with previous fertilization failure or oocyte activation deficiency [23, 31]. In our unselected cohort, calcium ionophore did not significantly increase fertilization but demonstrated a trend toward improved clinical outcomes. This echoes multi-center data showing that AOA is most effective when applied under specific indications such as globozoospermia or repeated ICSI failure [11]. Therefore, our results reinforce the idea that calcium ionophore AOA may improve clinical pregnancy rates in selected patient groups rather than universal application.

Piezoelectric activation facilitates sperm injection by reducing mechanical stress on the oocyte membrane, which may improve oolemma survival and promote fertilization [32]. However, the subsequent developmental competence of embryos may still depend on intrinsic oocyte quality, sperm factors, and culture conditions. The higher fertilization but similar pregnancy rates observed in our study suggest that piezoelectric may primarily optimize the initial fertilization step without fully overcoming downstream developmental bottlenecks.

Calcium ionophores mimic physiological calcium oscillations that follow sperm–oocyte fusion, thereby promoting activation. However, differences in ionophore type (ionomycin vs. A23187), exposure time, and laboratory protocols influence the magnitude of this effect [33, 34]. In our study, calcium ionophore was associated with more favorable trends in ongoing pregnancy and live birth (though not statistically significant), consistent with prior observations that proper Ca^{2+} signaling enhances developmental competence and implantation potential [25, 35].

From a clinical standpoint, our data suggest that ICSI+Piezoelectric may be useful in cases where maximizing fertilization and embryo availability is critical—for example, in poor responders or when oocyte numbers are limited. Calcium ionophore AOA, in contrast, may hold greater value in cases with suspected activation deficiency, as broad application to unselected populations may not consistently improve outcomes.

The numerically higher pregnancy and live birth rates observed in the calcium ionophore group, despite lower fertilization rates, may reflect differences in the quality and synchronization of oocyte activation. Chemical activation may induce a more sustained calcium signaling pattern in oocytes with intrinsic activation deficiency, potentially supporting downstream embryonic competence and implantation, even when fewer embryos are generated. In contrast, piezoelectric activation may efficiently trigger fertilization in a larger proportion of oocytes, including those with compromised developmental potential, thereby increasing embryo yield but not necessarily improving the proportion of embryos capable of achieving implantation and live birth.

This study has several limitations. First, although group sizes were balanced, the overall sample size may have been insufficient to detect modest but clinically relevant differences in live birth rates, increasing the risk of type II error. Although assisted oocyte activation techniques, including piezoelectric activation, have raised concerns regarding long-term safety, no increase in adverse obstetric or neonatal outcomes was observed in our cohort. However, given the limited sample size and follow-up period, larger studies with long-term follow-up are required to fully evaluate the safety profile of these techniques.Second, female age was slightly but significantly different between groups. Third, the groups were not randomized and were formed sequentially, which may introduce selection bias and temporal confounding. Although all patients were enrolled prospectively using predefined criteria and treated consecutively, unmeasured temporal

or clinical factors may have influenced the observed outcomes. Therefore, the results should be interpreted with caution .Finally, the study was conducted in a single center, which may limit generalizability across laboratories with different stimulation and culture protocols. Another significant limitation of this study is the absence of time-lapse morphokinetic assessment and preimplantation genetic testing (PGT-A) for aneuploidy, which could provide more detailed information about embryo developmental competence. Therefore, embryo quality in this study was assessed according to traditional morphological criteria.

Piezoelectric activation was associated with higher fertilization rates and improved early embryo development in ICSI cycles with severe male factor infertility. However, these improvements did not translate into statistically significant differences in clinical pregnancy or live birth rates. Larger, well-designed randomized controlled trials are required to determine whether the observed laboratory benefits of piezoelectric activation or calcium ionophore–induced artificial oocyte activation result in meaningful improvements in cumulative live birth outcomes.

Acknowledgements
None.

Authors' contributions
**Ayse Altun Akpinar: ** Conceptualization, Methodology, Data Curation, Writing – Original Draft, Visualization- **Burcin Karamustafaoglu Balci: ** Clinical Investigation-Supervision, Resources, Writing – Review & Editing- **Cigdem Celik: ** Project Administration, Writing – Review & Editing- **Busra Duymaz Edgu: ** Formal Analysis, Data Curation, Validation- **Cemil Akgul: ** Conceptualization, Methodology, Supervision, Writing – Review & Editing.

Funding
This study was conducted without any specific financial support from public, commercial, or non-profit funding agencies.

Data availability
The data that support the findings of this study are not publicly available due to restrictions on participant privacy but are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent about participating to the study and publication of their data was obtained from all participants prior to their inclusion in the study. Informed consent form was prepared based the documents that were submitted for and approved by ethics committee.

Consent for publication
All authors have read the final form of the manuscript and approved for its publication.

Competing interests
The authors declare no competing interests.

Received: 16 December 2025 / Accepted: 25 February 2026
Published online: 09 March 2026

References

1. Sfontouris IA, Nastri CO, Lima ML, Tahmasbpourmarzouni E, Raine-Fenning N, Martins WP (2021) Artificial oocyte activation to improve reproductive outcomes in ICSI cycles: A systematic review and meta-analysis. Hum Reprod Update 27(1):96–117
2. - Azil S, Mbaye MM, Louanjli N, Ghazi B, Benkhalifa M (2024) Phospholipase C zeta: a hidden face of sperm for oocyte activation and early embryonic development. Obstet Gynecol Sci 67(5):467–480
3. Krawetz SA (2005) Paternal contribution: new insights and future challenges. Nat Rev Genet 6:633–642
4. Sharma U, Conine CC, Shea JM, Boskovic A, Derr AG, Bing XY et al (2016) Biogenesis and function of tRNA fragments during sperm maturation and fertilization in mammals. Science 351:391–396
5. Aston KI, Conrad DF, Carrell DT (2012) Paternal influences on embryo quality. Reproduction 144:401–410
6. Tesarik J, Greco E, Mendoza C (2002) Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. Hum Reprod 17:1309–1314
7. Miller D, Brinkworth M, Iles D (2010) Paternal DNA packaging in spermatozoa: more than the sum of its parts? DNA Repair 9:1146–1157
8. Kashir J, Heindryckx B, Jones C (2010) Oocyte activation, phospholipase C zeta and human infertility. Hum Reprod Update 16(6):690–703
9. Mansour R, Fahmy I, Tawab N (2009) Electrical activation of human oocytes after intracytoplasmic sperm injection: A controlled randomized study. Fertil Steril 91(1):133–139
10. Campos P, de los Santos MJ, Meseguer M (2023) Artificial oocyte activation improves reproductive outcomes in cases of prior fertilization failure: A systematic review and meta-analysis. Front Endocrinol 14:1167894
11. Akashi K, Yamada M, Jwa SC, Utsuno H, Kamijo S, Hirota Y et al (2023) Artificial oocyte activation using Ca2 + ionophores following intracytoplasmic sperm injection for low fertilization rate. Front Endocrinol 9:14:1131808
12. Nomikos M, Sanders JR, Kashir J (2015) The role and mechanism of action of sperm PLCζ in mammalian oocyte activation. Biochem J 465(3):367–375
13. Saunders CM, Larman MG, Parrington J (2002) PLCζ: A sperm-specific trigger of Ca^{2+} oscillations in eggs and embryo development. Development 129(15):3533–3544
14. Göde F, Yılmaz M, Keskin T (2025) Comparison of calcium ionophore and piezo-electric activation in sibling oocytes: A prospective clinical study. J Assist Reprod Genet 42(6):891–899
15. Azil S, Kaarouch I, Montjean D, Pagé MHG, Cabry R, Louanjli N et al (2025) Effect of Sperm DNA Fragmentation and Chromatin Decondensation on PLCζ Efficacy in Infertile Patients. Curr Issues Mol Biol 47:707
16. Cavarocchi E, Drouault M, Ribeiro JC, Simon V, Whitfield M, Touré A (2025) Human asthenozoospermia: Update on genetic causes, patient management, and clinical strategies. Andrology 13(5):1044–1064
17. Montoya F, Bribiesca-Sánchez A, Hernández-Herrera P, Díaz-Guerrero DS, Gonzalez-Cota AL, Bloomfield-Gadelha H et al (2025) 3D + t Multifocal Imaging Dataset of Human Sperm. Sci Data 18(1):814
18. Kimura Y, Yanagimachi R (1995) Intracytoplasmic sperm injection in the mouse using a piezo-driven pipette. Biol Reprod 52:709–720
19. Caddy M, Popkiss S, Weston G, Vollenhoven B, Rombauts L, Green M et al (2023) PIEZO-ICSI increases fertilization rates compared with conventional ICSI in patients with poor prognosis. J Assist Reprod Genet 40(2):389–398
20. Miyara H, Morimoto Y, Yamasaki N, Shibahara H (2023) PIEZO-ICSI and clinical outcomes in fresh and frozen transfers: a comparative study. J Assist Reprod Genet 40:1919–1930
21. Yokota Y, Kato K, Ueno S, Uchiyama K, Okada H (2024) Comparison of clinical outcomes between conventional ICSI and piezo-ICSI in fresh and frozen embryo transfer cycles. Reproductive Med Biology 23:e12567
22. Kimura Y, Yanagimachi R (1995) Intracytoplasmic sperm injection in the mouse. Biol Reprod 53:855–862
23. Heindryckx B, De Sutter P, Gerris J, Dhont M, Van der Elst J (2008) Assisted oocyte activation in human oocytes with suspected activation deficiency. Hum Reprod 23:268–276
24. Takeuchi T, Neri QV, Katagiri Y, Rosenwaks Z, Palermo GD (2010) Effect of piezo-assisted ICSI on oocyte membrane integrity and fertilization outcome. Fertil Steril 94:1442–1446
25. Tesarik J, Sousa M, Testart J (1994) Human oocyte activation after ICSI: calcium oscillations and embryo development. Hum Reprod 9:511–518
26. Swann K, Lai FA (2016) Egg activation at fertilization by a soluble sperm protein. Physiol Rev 96(1):127–149

27. Eftekhar M, Janati S, Rahsepar M, Aflatoonian A (2013) Effect of oocyte activation with calcium ionophore on ICSI outcomes in teratospermia: A randomized clinical trial. Iran J Reprod Med 11(11):875–882
28. Ebner T, Moser M, Sommergruber M (2015) Artificial oocyte activation: a review of current practice and future prospects. Reprod Biomed Online 30(6):609–616
29. Yanagida K, Katayose H, Yazawa H, Kimura Y, Konnai K, Sato A (1999) Successful fertilization and pregnancy following ICSI with Piezo-micromanipulation. Hum Reprod 14(2):457–462
30. Heindryckx B, Van der Elst J, De Sutter P, Dhont M (2005) Treatment of oocyte activation failure after ICSI. Hum Reprod 20:2237–2241
31. Li S, Xiong F, Yu L, Hu J, Liu R (2022) Assisted oocyte activation with calcium ionophore improves outcomes after ICSI: a systematic review and meta-analysis. Front Physiol 13:751905
32. Yanagida K, Katayose H, Yazawa H, Kimura Y, Sato A, Yanagimachi R (2001) Successful fertilization and pregnancy following ICSI with piezo micromanipulation. Hum Reprod 16:1464–1469
33. Shebl O, Ebner T, Oppelt P, Mayer RB (2023) Artificial oocyte activation with ionomycin vs. A23187: a prospective comparison of developmental kinetics. J Assist Reprod Genet 40:2335–2347
34. Zheng X, Zhang Y, Wang Z, Sun Y (2021) Calcium ionophore (A23187) and embryo development/clinical outcomes: narrative review and meta-analytic evidence. Front Endocrinol 12:692082
35. Borges E, de Almeida Ferreira Braga DP, Setti AS, Figueira RCS, Iaconelli A (2009) Artificial oocyte activation with calcium ionophore in ICSI cycles: a randomized trial. Hum Reprod 24:1359–1366

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.