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ORIGINAL ARTICLE

The Use of Artificial Oocyte Activation as an Adjunct in Addressing Fertilisation Failures among Women Undergoing In Vitro Fertilisation Cycles: A University Cohort Study

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ABSTRAK

Pelaksanaan suntikan sperma ke dalam sitoplamik (ICSI) terutamanya kepada rawatan faktor kemandulan lelaki telah diperkenalkan namun masih ada sekurang-kurangnya 1-5% kegagalan persenyawaan berterusan disebabkan pengaktifan oosit yang lemah. Oleh itu, kami melakukan kajian kohort prospektif di Pusat Reproduktif Termaju, Hospital Canselor Tuanku Muhriz, Universiti Kebangsaan Malaysia untuk menilai peranan pengaktifan oosit buatan (AOA) dalam meningkatkan kadar persenyawaan melalui penggunaan kalsium ionofor GM508. Kajian ini melibatkan seramai 43 wanita yang didapati mempunyai kadar persenyawaan rendah (LFR) atau kegagalan keseluruhan persenyawaan (TFF) dengan membandingkan kitaran indeks persenyawaan in vitro (IVF) mereka dengan kitaran IVF-AOA berikutnya. Kami mendapati bahawa kebanyakan peserta merupakan wanita yang lebih berusia, dengan purata umur 38.44 tahun dan majoriti mengalami subfertiliti primer yang kebanyakannya berpunca daripada faktor lelaki. Penemuan kami mendapati peningkatan dalam kadar persenyawaan (FR) dengan peningkatan selanjutnya dalam kadar belahan embrio dan kadar kualiti embrio terbaik (TQE). Selain itu, kadar TQE juga didapati lebih baik dalam kohort TFF berbanding kohort LFR. Secara keseluruhan, penemuan kajian ini menyerlahkan potensi AOA untuk meningkatkan FR dan hasil embriologi. Walau bagaimanapun, kajian lanjut yang melibatkan sampel yang lebih besar dan pelbagai pusat diperlukan untuk pengesyoran yang lebih luas.

Kata kunci: *Kadar persenyawaan; kegagalan persenyawaan; pengaktifan oosit buatan; suntikan sperma ke dalam sitoplamik*

ABSTRACT

The current implementation of intracytoplasmic sperm injection (ICSI) has mainly been for male factor, but 1-5% fertilisation failure persists due to poor oocyte activation. Thus, we performed a prospective cohort study at the Advanced Reproductive Centre, Hospital Canselor Tuanku Muhriz, Universiti Kebangsaan Malaysia to assess the role of artificial oocyte activation (AOA) in improving the fertilisation

rates via calcium ionophore GM508. Our study included a total of 43 women diagnosed with low fertilisation rate (LFR) or total fertilisation failure (TFF) by comparing their index in vitro fertilisation (IVF) cycles with subsequent IVF-AOA cycles. We found that, most women were older, 38.44 years old, with a majority experiencing primary subfertility, primarily due to male factors. Our findings found an increase in fertilisation rates (FR) with further improvements in cleavage rates, blasturation rates and top embryo quality rates (TQE). Furthermore, TQE was also better in the TFF cohort compared to the LFR cohort. Overall, our findings highlight the potential of AOA to improve FR and embryological outcomes; however, further search with larger and multicenter studies is necessary for broader recommendations.
Keywords: Artificial oocytes activation; fertilisation rates; fertilisation failure; intracytoplasmic sperm injection

INTRODUCTION

Intracytoplasmic sperm injection (ICSI) has significantly improved in vitro fertilisation (IVF) by increasing fertilisation rates, particularly for couples facing sperm-related issues (Zhu et al. 2024). Since its introduction, ICSI has shown greater success in fertilisation compared with conventional techniques. According to the Vienna consensus, the normal fertilisation rate for ICSI is approximately 60-80% across all cycles performed worldwide (ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine 2017). However, fertilisation failure after ICSI can still occur in at least 5% of IVF cycles. Investigations into these failures have indicated that the underlying cause may be oocyte activation deficiency (OAD) (Kashir et al. 2022).

Molecularly, the release of intracellular calcium primarily regulates oocyte activation. Mature oocytes are arrested in the metaphase II (MII) stage and complete meiosis only when the sperm protein phospholipase-C-Zeta (PLCζ) enters the oocyte cytoplasm during gamete fusion (Sugita et al. 2024). PLCζ then triggers characteristic cytosolic Ca^{2+} oscillations that activate a complex signalling cascade essential for resuming meiosis, extruding the second polar body, forming pronuclei and exocytosing cortical granules (Chen et al. 2024). If these Ca^{2+} oscillations do not occur due to factors related to either the oocyte or sperm, insufficient oocyte activation can lead to fertilisation failure. To address this issue, scientists have developed various strategies, including artificial oocyte activation (AOA), which is now widely implemented in clinical practice using several techniques (Chen et al. 2024; Kashir et al. 2022). These methods include the application of chemical agents, such as calcium ionophores (e.g., ionomycin and calcimycin) and strontium chloride ($SrCl_2$), as well as mechanical and electrical stimulation (Gode et al. 2025; Vanden Meerschaut et al. 2014b) (Figure 1). Chemical agents are the most commonly used and effective for activating oocytes. Evidence suggests that AOA can improve fertilisation rates by at least 22% in women with a history of previous fertilisation failures (Wang et al. 2021).

A recent cumulative meta-analysis of 14 studies concluded that AOA using any calcium ionophore significantly increases live birth rates (LBRs) (Murugesu et al. 2017). Additionally, a similar study revealed that subgroup analysis showed AOA with calcium ionophores significantly increased LBR in patients with a history of fertilisation failure or low fertilisation rate (LFRs) (Ruan et al. 2023). The implementation of AOA, particularly with calcium ionophores, has been well established in terms of safety. Calcium ionophores interact with calcium molecules to form complexes at the lipid bilayer of the cell membrane. As a result of their hydrophobic properties, these ionophores can transport calcium across the membrane and release into the cytosol. Consequently, ionophores may not penetrate the oocyte, which may explain the absence of observable effects on chromosomes, gene expression (compared with conventional

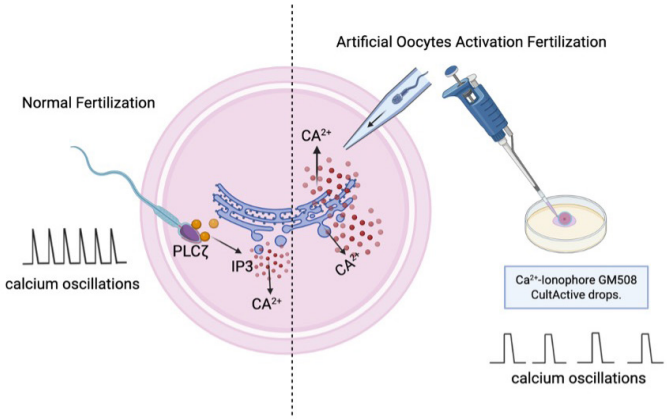

FIGURE 1: Comparison between physiological oocytes and artificial oocytes activation with calcium oscillations.
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IVF) or morphokinetics (Xu et al. 2021).

Despite the limited data on the relationship between AOA and obstetric or neonatal outcomes, a retrospective study found no congenital disabilities among children conceived through ICSI and AOA (Long et al. 2020). Furthermore, evidence shows no differences in the growth and health parameters of infants born via the two AOA protocols compared with those born through ICSI (Miller et al. 2016). A recent study evaluated the long-term neurodevelopmental outcomes of children aged 3-10 years who were born after assisted reproductive technology or AOA. Assessments in neurodevelopment, intelligence, language and social communication indicated that all parameters were within the expected range (Vanden Meerschaut et al. 2014a). Therefore, health concerns regarding the ethical implications and safety of AOA have been adequately addressed by demonstrating that the method is safe and compliant with accepted ethical standards.

In Malaysia, AOA has been practiced for several years, primarily in IVF settings; however, local evidence remains limited. Most centers implement AOA based on their interests, as proper clinical evaluations are pending. Consequently, we evaluated the overall IVF outcomes following AOA utilisation by comparing them with the women's previous standard cycles. Thus, our study help in formulating effective local recommendations to enhance the implementation of AOA in current IVF practices in Malaysia.

MATERIALS AND METHODS

Study Recruitment

This prospective observational study was conducted at the Advanced Reproductive Centre at Hospital Canselor Tuanku Mukhriz, Universiti Kebangsaan Malaysia (UKM). We obtained ethical clearance from the UKM Research Ethics Committee (JEP-2023-886) and informed consent were obtained prior to participant recruitment. Data on women undergoing IVF cycles were collected from January 1, 2024, to December 31, 2024. The study focused on women identified as having experienced fertilisation failure, which was categorised as either LFR or total fertilisation failure (TFF), based on their previous ICSI cycles. Only women undergoing their second or third ICSI cycles (ICSI-AOA) were included in this study. LFR is defined as a fertilisation rate of less than 30%, whereas TFF is defined as no normal or any fertilisation achieved in a prior IVF cycle (Wang et al. 2022). We excluded couples with male factors that required surgical sperm retrieval (SSR) from this study. Previous cycles that did not utilise AOA served as a control group. The interval between the two stimulation cycles in

this study did not exceed 12 months. For analysis, we divided our participants into two subgroups: those with TFF and those with LFR. We compared the fertilisation rates and the development of top-quality embryos from the ICSI-AOA cycles with those from the participants' previous ICSI cycles, focusing on embryological outcomes of interest. Based on a similar study by Murugesu et al. (2017), at least 44% of the improvement in fertilisation was following AOA. Therefore, P0 was taken as 0.56 (failure of fertilisation) based on this study. This value represented the baseline failure rate of fertilisation. P1 was set at 0.5, aiming for at least a 50% improvement in fertilisation following AOA. This value represented the expected failure rate of fertilisation after the intervention. Otherwise, m was a comparison 1:1, and the power was 80%. Therefore, our sample size was generated based on the PS Sample Size calculation, with 18 participants in one arm, totalling 36. We added 10% of drop-off participants, so our total sample size was 40. All women participating in the study underwent controlled ovarian hyperstimulation (COH), mainly using an antagonist protocol. The choice of gonadotropin was based on the clinicians' preferences, and may be combined with oral agents, such as clomiphene citrate, an aromatase inhibitor or trigger agents, as needed.

Controlled Ovarian Hyperstimulation, Oocyte Retrieval and Artificial Oocyte Activation Procedure

Our standard COH protocol employed a combination of gonadotropins, specifically Folliculin® (BSV, Mumbai, India) or follitropin alfa (Gonal-F®, Merck, Darmstadt, Germany). We also included highly purified menotrophin (Humog®, BSV, Mumbai, India). The initial dosage was set at 225 IU daily, with the option of gradually increased it to 375 IU based on the observed response in follicular growth. Once the follicles reached a size of 10-12 mm, we administered an additional antagonist injection, either Cetrotide® (Merck, Darmstadt, Germany) or a GnRH antagonist (BSV, Mumbai, India), to prevent premature ovulation. For our minimal stimulation protocol, we started with oral ovulation induction using either an aromatase inhibitor or clomiphene citrate during the first 5 days. Thereafter, we administered highly purified menotrophin (Humog®, BSV, Mumbai, India) at a dosage of 225 IU on alternating days (specifically on days 3, 5 and 9) throughout the ovarian stimulation phase. When the follicles reached a diameter of 10-12 mm, we administered an additional antagonist injection, such as Cetrotide® (Merck, Darmstadt, Germany) or Goserelin® (BSV, Mumbai, India), to prevent premature ovulation. In our practice, when two or more dominant follicles reached a mature size of 18-20 mm, we administered the triggering agent (LH/hCG). The triggering agent may be Ovidrel® (Merck, Darmstadt, Germany), Hucog® (BSV, Mumbai, India), or Decapeptyl® (Ferring, Saint-Prex, Switzerland), depending on the clinician's preference. A dual trigger involving these options may also be employed at the clinician's discretion. OR was then performed at least 34-36 hours after the triggering agent was administered under sedation, in accordance with local protocols. The OR procedure was conducted using a double-lumen needle (Cook® USA) with a pressure setting of 120-140 mmHg. The semen sample was collected fresh in a sterile environment after a minimum abstinence period of 2 days on the morning of the ICSI procedure. After oocyte retrieval (OR), the oocytes were incubated in a standard incubator at 37˚C, 6% CO_2 and 5% O_2 for at least 4 h prior to denudation. Once denudation was complete, all metaphase II (MII) oocytes underwent standard ICSI procedures. AOA was performed according to the manufacturer's instructions and modified by our local protocol. In brief, after 30 minutes of ICSI, all oocytes were exposed to an AOA medium containing 30 µL of calcium ionophore (Cult-Active, Gynemed, Sierksdorf, Germany) for at least 15 minutes. The oocytes were then rinsed with medium (G-IVF, Vitrolife Sweden AB, Gothenburg, Sweden) in at least three cycles and finally incubated in embryo culture medium (GTL, Vitrolife Sweden AB, Gothenburg, Sweden). Subsequently, fertilisation was observed and recorded at least 18 h post-ICSI by using a time-

lapse embryoscope. Only oocytes that exhibited two clear pronuclei (2PN) were considered to have undergone normal fertilisation.

Embryo Quality Assessment

The outcomes of embryology were assessed based on either the cleavage stage (day 3 embryos) or the blastocyst stage (day 5 blastocysts), determined by counting the total number of normally fertilised oocytes. We evaluated the quality of day 3 (D3) embryos according to the ESHRE Istanbul Consensus Workshop recommendations (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology 2011). In summary, good embryo quality (EQ) was defined as having less than 10% fragmentation and no multinucleation. Fair EQ was characterised by 10-25% fragmentation without multinucleation. Poor EQ was indicated by severe fragmentation, which may occur in conjunction with multinucleation. To score day 5 (D5) blastocysts, we used a modified version of the Gardner scoring system adapted to our local protocol (Pierson et al. 2023). This system assesses cellular expansion on a scale of 1 to 6. A score of 6 represented the most expanded state (with 100% blastocoel and fully hatched), and 1 indicated a thick zona pellucida (ZP) with a blastocoel comprising less than 50%. We also evaluated the quality of the inner cell mass (ICM) and the trophectoderm (TE), both of which were rated as A, B or C. For the ICM, A denoted good quality with tightly packed cells, B indicated fair quality with loosely grouped cells and C signified poor quality with very few disorganised cells. For the TE, A represented many cells forming a cohesive epithelium, B denoted a few cells forming a loose epithelium and C indicated very few large cells. In our study, top-quality embryos (TQEs) were defined as good embryos based on the D3 evaluation.

Statistical Analysis

Sudy findings are presented as medians with interquartile ranges (IQR). The Shapiro–Wilk test assessed normality of continuous data. The Student's t-test was used for normally distributed variables, while the Mann–Whitney U or Wilcoxon signed-rank test was used for non-normally distributed data. The Kruskal–Wallis test was used to compare more than two independent groups. Spearman's rank correlation coefficient assessed correlations between non-normally distributed variables. Fisher's exact test was used for categorical variables. Statistical significance was defined as a two-sided p-value < 0.05. Analyses were conducted using Statistical Package for the Social Science, version 24.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Demographic

A total of 43 women with a history of suboptimal fertilisation in previous ICSI cycles were included in the study cohort and subsequently sub-analysed according to causes of fertility and an indication of AOA (Figure 2). The average age of these participants was 38.44 $\pm$ 3.95 years, with a significant majority experiencing primary infertility, accounting for 67.4% of the cohort. The mean duration of infertility prior to the commencement of fertility treatment was 8.14 $\pm$ 4.11 years. Regarding the underlying causes of infertility, 44.2% were attributed to female factors, 34.9% to male factors, 11.6% to a combination of male and female factors and 9.3% were classified as having unexplained infertility. Most women in this cohort were classified as overweight (based on BMI), with a mean of 26.71 kg/m . Furthermore, the majority exhibited diminished ovarian reserve (DOR), as evidenced by an average anti-Müllerian hormone level of 8.41 pmol/L. Notably, at least 34 women (79.1%) had undergone AOA because of a prior LFR, whereas nine women (20.9%) received AOA following TFF (Table 1).

In Vitro Fertilisation, Fertilisation and Top-Quality Embryos Outcome

Both cohorts followed similar COH protocols.

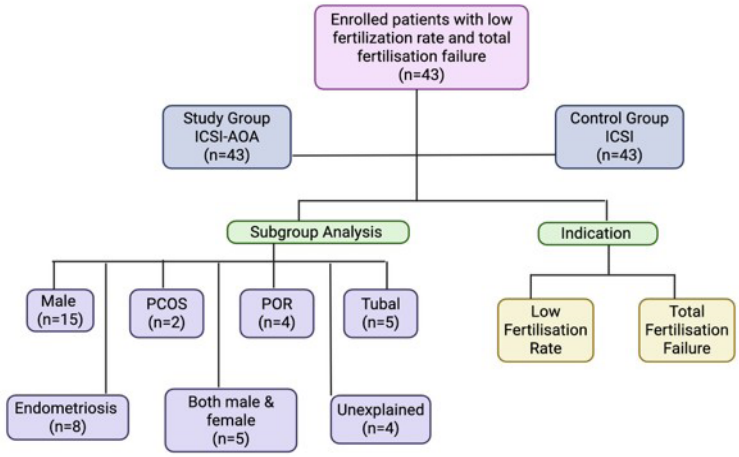

FIGURE 2: The study flow

TABLE 1: Demographic profile

Parameter	mean ± SD
Age (years old)	38.44 ± 3.95
BMI (kg/m^2)	26.71 ± 5.47
AMH (pmol/L)	8.41 ± 6.68
Duration of infertility (years)	8.14 ± 4.11
Type of infertility	*n* (%)
Primary	29 (67.40)
Secondary	14 (32.50)
Indication	n (%)
Female	19 (44.20)
Male	15 (34.90)
Both	5 (1.60)
Unexplained	4 (9.30)
Indication for AOA	n (%)
Low Fertilisation rate	34 (79.10)
Total fertilisation failure	9 (20.90)

AMH: Anti mullerian hormone; AOA: Artificial oocyte activation; BMI: Body mass index

Overall, we observed significant differences in several parameters between the control and AOA groups, including the number of oocytes retrieved, number of 2PN (fertilised) oocytes, number of cleavage embryos, number of blastocysts and rate of TQEs. However, we found no significant differences in the number of matured oocytes (MII) between the two groups despite a notable difference in the number of retrieved oocytes. Additionally, the fertilisation rate, cleavage rate, blastocyst formation rate and top EQ rate were significantly higher in the AOA group than in the control group ($p < 0.001$). When examining fertilisation rates and causes of infertility, we found that fertilisation rates across all causes significantly increased compared with previous ICSI cycles, except for women with PCOS. Although we observed an overall improvement in the top EQ rate, this improvement was not statistically significant. Regarding fertilisation rates and indications for AOA, we noted a significant enhancement in fertilisation rates for the LFR and TFF groups. However, when evaluating the top EQ rate, a significant improvement was observed only in the TFF group following AOA (Table 2).

Subgroup Cause of infertility and the Indication of AOA

Subgroup analysis was conducted based on the cause of infertility and the resulting fertilisation rates. The causes of infertility were categorised into six groups: male factors, endometriosis, combined male and female factors, polycystic ovary syndrome (PCOS), poor ovarian reserve (POR), tubal factors and unexplained factors. Fertilisation rates in each category showed a significant increase compared to their

TABLE 2: Overall in vitro fertilisation outcome

Parameter	ICSI group (control)	ICSI AOA group	*t*-value	*p*-value
No of women	43	43		
No of oocytes obtained	8.05 ± 4.82	9.74 ± 7.64	2.657	0.011*
No of MII oocytes	6.56 ± 3.54	7.37 ± 5.82	1.591	0.119
No of oocytes fertilised	1.77 ± 1.29	4.72 ± 4.08	6.111	<0.001*
Fertilisation rate (%)	26.95 (76/282)	64.04 (203/317)	11.484	<0.001*
No of cleaved embryo	1.37 ± 1.02	4.33 ± 3.40	7.176	<0.001*
Cleavage rate (%)	77.6 (59/76)	91.6 (186/203)	4.970	<0.001*
No of blastocyst	0.72 (±0.815)	4.21 (±3.507)	4.379	<0.001*
Blastocyst formation rate (%)	34.21 (26/76)	68.47 (139/203)	4.42	<0.001*
No of top-quality embryo	Day 3:1.00 (±0.866)	Day 3:1.20 (±1.033)		
	Day 5: 0.58 (±0.614)	Day 5:2.32 (±1.66)		
Top quality embryo rate (%)	47.4 (28/59)	48.9 (91/186)	2.385	0.022*

AOA: Artificial oocyte activation; ICSI: Intracytoplasmic sperm injection; MII: Metaphase II; No: Number

corresponding previous ICSI cycles, except for patients with PCOS (Figure 3a). Interestingly, despite the overall improvement in the quality of top embryos, no significant difference was observed in the subgroup analysis (Figure 3b). Additionally, when analysing the reasons for advanced ovarian age (AdOA), significant improvements in fertilisation rates were noted in both the LFR and TFF groups (Figure 3c). However, when the analysis focused on top embryo quality, only the TFF group demonstrated a significant improvement (Figure 3d).

DISCUSSION

Our study confirmed that AOA which utilises a calcium ionophore, yielded positive outcomes for women experiencing fertilisation failure. We observed that the majority of women with TFF achieved better embryological results following the ICSI-AOA procedure compared with their earlier standard ICSI cycles. However, our findings contradicted existing evidence suggesting that AOA enhances fertilisation rates and promotes the development of high-quality embryos, particularly in cases of prior poor fertilisation outcomes commonly observed in assisted reproductive technologies (Nicholson et al. 2024; Wang et al. 2021).

Our primary focus was to assess the improvements in fertilisation rates and the subsequent development of TQEs available for transfer after AOA. Overall, our results indicated that AOA significantly improved fertilisation rates and increased the availability of TQEs in instances of prior LFRs and TFF. Specifically, our findings demonstrated that AOA treatment resulted in notable improvements in fertilisation rates, embryo cleavage rates, blastocyst formation rates and the generation of TQEs per woman compared with their previous ICSI cycles. These results were consistent with established evidence that noted similar outcomes following ICSI-AOA (Nicholson et al. 2024; Ruan et al. 2023; Wang et al. 2021).

Our findings support the current data that AOA enhances the fertilisation rate and the developmental potential of embryos, potentially leading to an increased yield of high-quality embryos (Nasr-Esfahani et al. 2010; Ruan et al. 2023; Vanden Meerschaut et al. 2014b; Zhu et al. 2024). Our results indicated that the fertilisation rate in the AOA group exceeded that of the standard ICSI method by more than

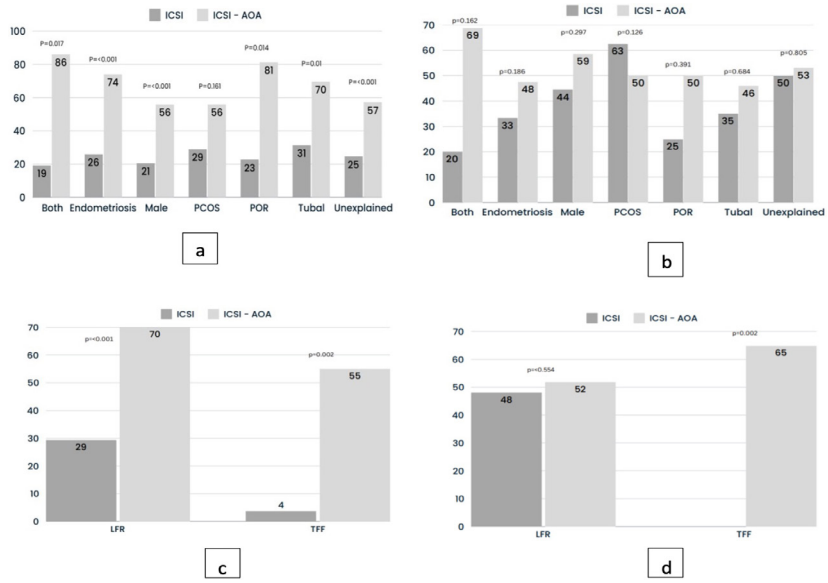

FIGURE 3: Sub-analysis comparison; (a) Comparison of fertilization rates between AOA subgroups and control groups; (b) Comparison of the top-quality embryo rate between AOA subgroups and control groups; (c) Comparison of fertilisation rates between AOA subgroups and control groups based on the indication for AOA; (d) Comparison of the top-quality embryo rate between AOA subgroups and control groups based on the indication for AOA

50%. Scholars attributed this enhancement in fertilisation rates to molecular mechanisms; specifically, AOA induces calcium release and simulates natural oscillation, facilitating improved fertilisation outcomes that mirror those seen in women without activation defects (Sugita et al. 2024). However, our findings also indicated that AOA did not enhance fertilisation outcomes in women with PCOS. The majority of evidence suggested that oocytes from women with PCOS are affected by a variety of intrinsic and extrinsic factors, ultimately resulting in reduced oocyte quality and developmental competence (Qiao & Feng 2011; Urman et al. 2004; Yan et al. 2024). The inherent defects in oocyte quality, impaired cumulus-oocyte complex function, disrupted follicular microenvironment and hormonal imbalances associated with PCOS can lead to undue AOA-related complications, contributing to ongoing fertilisation failures despite the application of ICSI with AOA (Yan et al. 2024).

Since its introduction, AOA has been linked to many cases of subfertility due to oocyte activation failure, primarily resulting from sperm factors such as globozoospermia or severe male infertility (Nasr-Esfahani et al. 2010; Nicholson et al. 2024). The findings were highlighted in our study, as our male factor cohort was associated with elevated fertilisation rates following AOA-ICSI. Although our TQE did not significantly improve, multifactorial confounding was found to influence overall embryo formation. For example, increased oxidative stress, as well as genetic and epigenetic factors, influences overall embryological and developmental potential. Oxidative stress can impair mitochondrial function and energy metabolism involved in embryo development, but genetic defects in sperm (PLCZ) mutations may not be fully mitigated by AOA despite successful fertilisation (Wang et al. 2025). Established studies on new sperm-related oocyte activators have identified the post-acrosomal WW domain-binding protein (PAWP), located in the sperm head, which

facilitates fertilisation by triggering calcium oscillations in the oocyte (Nakai et al. 2020; Nomikos et al. 2014). To address cases where AOA has failed, advancements in reproductive technologies have introduced methods such as ICSI combined with the microinjection of mRNA PLCZ and recombinant active PLCZ protein (Saleh et al. 2024). The formation of TQEs is a multifaceted process influenced by biological, environmental, and procedural factors.

The characteristics of oocytes and sperm are equally crucial for successful embryo development (Smith & Russell 2022). Accordingly, our results showed no association between the top EQ rate and the causes of infertility. This finding may suggest that oocytes activation deficiency is not the sole reason for poor embryo development; other contributing factors may include high sperm DNA fragmentation and potential oocyte abnormalities in structural proteins, mitochondria, or transcription factors, all of which can contribute to poor EQ (Vahedi Raad et al. 2024). AOA alone cannot resolve all these issues.

Additionally, advanced maternal age and DOR did not show significant differences in top EQ rates, likely because oocyte activation failure is not the primary cause of fertilisation challenges. Ultimately, age-related defects affect mitochondrial DNA and gene regulation, leading to fertilisation failure that is independent of the AOA mechanism (Druzhyna et al. 2008). Although AOA offers numerous benefits, its safety requires further investigation. Currently, most experts agree that AOA does not appear to increase the risks of chromosomal abnormalities or congenital disabilities during pregnancy. A published meta-analysis revealed no significant differences in rates of congenital disabilities, birth weight, gestational age, preterm birth rates, early neonatal mortality or fetal sex ratios among infants conceived through IVF utilising AOA-ICSI (Long et al. 2020; Miller et al. 2016; Vanden Meerschaut et al. 2014a). However, there is a substantial gap in research regarding the effects of AOA on gene expression and epigenetics in early human embryos (Dvoran et al. 2022). Therefore, additional studies are essential to evaluate the overall outcomes for offspring conceived via AOA-ICSI.

Our findings suggested that AOA can improve treatment outcomes for women experiencing fertilisation failure and serves as a valuable adjunct in assisted reproduction. However, this study was limited by its small sample size and the fact that it was conducted at a single center. By comparing cycles between sisters in the control and intervention cohorts, we were able to rigorously evaluate the efficacy of AOA in relation to ICSI within our specific regional framework. This analysis provides insights into the localised outcomes of AOA and may guide future clinical recommendations. Nevertheless, the comparison between the cycles of sisters in the control and intervention groups allowed us to accurately assess the benefits of AOA in the ICSI procedure within our local situation. This reflects the AOA outcomes specific to our region and may guide clinical recommendations.

CONCLUSION

In conclusion, our study provides a foundation for future research on AOA-ICSI. It highlights the need for a multicenter cohort study with a large sample size to clarify further and confirm the overall benefits of AOA in the field of IVF mainly in fertilisation failure and overall embryo quality outcome.

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Ethics statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Human Ethics Committee of Faculty of Medicine, UKM (JEP-2023-886) and registered as FF-2024-017.

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