Artificial Oocyte Activation (AOA) Following Intracytoplasmic Sperm Injection (ICSI)

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Scientific group of Artificial Oocyte Activation Guideline

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Abbreviation

AOA: Artificial oocyte activation

ICSI: Intracytoplasmic sperm injection

IVF: in vitro fertilisation

OADs: oocyte activation deficiencies

PICO: Population, Intervention, Comparison and Outcomes

TFF: total fertilisation failure

Scope

This guideline primarily focuses on the recommendations for evaluating the efficiency and safety of Assisted Oocyte Activation (AOA) in Intracytoplasmic Sperm Injection (ICSI) to attain the most favourable reproductive and offspring health results. Additionally, it intends to analyse various evidence-based protocols and techniques that can be employed during AOA in ICSI. Additionally, it scrutinises numerous procedures that ought to be abandoned due to the lack of evidence substantiating their efficacy.

Executive Summary:

- IVF facilities could consider offering AOA for patients with previous no or low fertilisation, defined as lower than 30% (conditional recommendation).
- AOA could be considered after proper counselling and well-informing the patients of globozoospermia cases (conditional recommendation).
- IVF facilities should NOT recommend AOA for the general ICSI population (conditional recommendation against).
- IVF facilities should NOT recommend AOA for improving embryo quality (conditional recommendation against).
- IVF facilities should NOT recommend AOA for improving embryo development and blastocyst formation (conditional recommendation against).
- IVF facilities should NOT recommend rescue AOA either hours or on day 1 after ICSI (conditional recommendation against).
- IVF facilities could consider sibling-oocyte-split if AOA is offered for patients of previous low or no fertilisation, given the scarce long-term safety data (good practice statment).
- Except for globozoospermia cases, IVF facilities should NOT recommend AOA for ICSI cycle with male factor infertility and no history of no or low fertilisation (conditional recommendation against).
- Except for the above recommendations, AOA should only be considered within a research context without charging the patients and after proper counselling and written consent (good practice statment).

Introduction

Intracytoplasmic sperm injection (ICSI) is a widely employed fertility treatment technique utilised in clinics worldwide. The process entails the meticulous selection of a solitary sperm cell and its direct injection into an egg cell, also known as an oocyte. For the past thirty years, a small number of highly trained and skilled embryologists specialising in assisted reproductive technologies have manually performed the complex ICSI procedure [1].

ICSI has shown to be highly effective, with fertilisation rates reaching up to 70%. However, a small percentage of cycles (1-5%) may encounter either complete failure of fertilisation or low fertilisation rates (<30%). This phenomenon emphasises the intricate nature of fertilisation and the influence of multiple factors on the outcomes of intracytoplasmic sperm injection (ICSI), which justifies the need for additional research to enhance protocols [2].

Oocyte activation deficiencies (OADs) have been identified as the main cause of total fertilisation failure (TFF) after ICSI. The deficiencies in question are multifactorial, meaning they can arise from various causes, such as inherent abnormalities in either the spermatozoon or the oocyte [2, 3].

Oocyte activation is a complex series of molecular events that occur when sperm enters the oocyte. This process leads to periodic releases of calcium from the endoplasmic reticulum [4-6]. Artificial oocyte activation (AOA) is a treatment that aims to trigger calcium release after ICSI artificially. This is done using different methods such as chemicals, mechanical means, or electrical stimulation [7]. Calcium ionophores, such as ionomycin and calcimycin, are frequently used chemical agents for AOA. They work by causing the release of calcium from the endoplasmic reticulum and promoting the entry of calcium from outside the cell [2, 3, 8]. AOA is primarily utilised in patients who experience fertilisation failure or a reduced fertilisation rate following ICSI.

Multiple studies have shown that AOA effectively enhances the fertilisation rate in such patients [9-12]. In addition, it has been discovered that augmented calcium signalling plays a vital role in causing changes in fertilised oocyte nuclei and cytoplasm. This process triggers oocyte activation and the initial stages of embryogenesis [7, 8]. Furthermore, evidence indicates that AOA may improve the growth and development of embryos [10, 13, 14]. This guideline aims to aid embryologists in making informed decisions regarding the performance of AOA procedures.

THE TARGET AUDIENCE

This guideline is specifically aimed at embryologists who perform IVF/ICSI procedures. Additional population targets encompass reproductive medicine specialists, gynaecologists, infertile couples, and other relevant professionals.

Methods

This guideline document was generated using a systematic review and meta-analysis of the relevant literature, followed by evidence grading.

1. Registration, reporting, and patient and public involvement

Following the preregistration of the entire project on creating Egyptian IVF laboratory guidelines with the Centre for Open Science (https://doi.org/10.17605/OSF.IO/23KBU) on 21 February 2024, we proceeded to implement the appropriate methodology for studying the impact of AOA on reproductive outcomes as outlined in the guidelines. We documented the findings in accordance with the prescribed protocols. We did not involve patients or the public in any aspect of the design or implementation of this review.

2. Literature search strategy and selection

We formulated an all-encompassing search plan for the pertinent databases: Cochrane Library, PubMed, and Scopus. The search encompassed keywords and different forms of terms associated with 'AOA', 'sperm abnormalities', 'assisted reproduction', 'IVM oocytes', 'ART', 'in vitro fertilisation', 'Intracytoplasmic sperm injection', 'testicular sperm', 'fertilisation failure', 'embryo development', 'pregnancy outcomes', 'embryo implantation', 'live birth', 'neonatal outcomes', and 'birth defects'. We systematically searched for studies examining the correlation between AOA and outcomes of assisted reproduction. Our search was limited to studies conducted in English and involving human subjects. We conducted a comprehensive examination of the references cited in the selected reviews. We conducted a literature search until the date of March 1, 2024. Information regarding the literature search, reviewers, and other relevant details will be documented in separate publications that undergo peer review. The reviewers convened a meeting to establish a standardised selection strategy. Once the eligible citations were identified as duplicates, the full texts were thoroughly examined, and relevant data was extracted.

3. Synthesis of the evidence

We exclusively incorporated primary studies that contained quantitative data. We employed a random-effects model to calculate the magnitude of the association using the odds ratio (OR) across all studies. We utilised the Hartung–Knapp–Sidik–Jonkman method, which is regarded as superior when heterogeneity exists [14].

We classified the evidence using the GRADE (http://www.gradeworkingroup.org)

framework, along with a more extensive set of criteria that had been previously published [15, 16]. The WHO Handbook for Guidelines employs the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) approach to evaluate the quality of evidence, create recommendations, and present them [17]. The World Health Organisation (WHO) utilises GRADE methods due to their status as globally recognised standards for generating clear and consensus-based recommendations. Comprehensive information regarding GRADE can be found on the following websites:

- *GRADE working group: http://www.gradeworkingroup.org*
- *GRADE online training modules: http://cebgrade.mcmaster.ca/*
- *GRADE profile software: http://ims.cochrane.org/revman/gradepro*

Table 1 Quality of Evidence in GRADE

Quality level	Definition
High	We are very confident that the true effect lies close to that of the estimate of the effect.
Moderate	We are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low	Our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low	We have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.
GRADE: Grading of Recommendations Assessment, Development and Evaluation.	

Table 2 Significance of the four levels of evidence

Quality	Definition	Implications
High	The guideline development group is very confident that the true effect lies close to that of the estimate of the effect	Further research is very unlikely to change confidence in the estimate of effect

Moderate	The guideline development group is moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different	Further research is likely to have an important impact on confidence in the estimate of effect and may change the estimate
Low	Confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the true effect	Further research is very likely to have an important impact on confidence in the estimate of effect and is unlikely to change the estimate
Very low	The group has very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of the effect	Any estimate of effect is very uncertain

Recommendation

1. AOA for patients with previous no or low fertilisation

IVF facilities could consider offering AOA for patients with previous no or low fertilisation, defined as lower than 30% (low-grade evidence—conditional recommendation) [10, 11, 18-29].

The recommendation for IVF clinics to contemplate providing AOA to patients who have experienced no or limited fertilisation rates, specifically below 30%, is grounded on a careful evaluation of the potential advantages and disadvantages. Despite the limited quality of evidence, certain studies suggest that the use of AOA may enhance fertilisation rates in patients who have encountered low or no fertilisation after ICSI [10, 11, 21, 24, 28]. This potential advantage could help patients who have experienced previous low fertilisation, as enhancing fertilisation may increase the likelihood of successful clinical outcomes. Nevertheless, the available evidence is constrained by the use of small sample sizes, diverse study designs, and potential biases, all of which collectively diminish the strength and reliability of the evidence [6]. Hence, due to the inadequate quality of evidence, patients with low or no fertilisation should receive comprehensive information regarding the potential advantages and uncertainties linked to AOA, enabling them to make well-informed decisions. Clinicians should apply their professional expertise and take into account the unique circumstances of each patient, including their specific history of fertilisation and overall reproductive well-being, in order to determine whether to provide AOA. Implementing AOA requires allocating extra resources. Thus, IVF facilities should carefully evaluate these resource factors in relation to the potential advantages of enhanced fertilisation rates for this specific group of patients.

2. AOA for globozoospermia cases

AOA could be considered after proper counselling and well-informing the patients of globozoospermia cases (low-grade evidence—conditional recommendation) [30, 31].

The recommendation to consider AOA for patients with globozoospermia, after providing thorough counselling and informing the patients adequately, arises from the unique difficulties presented by this uncommon condition. Globozoospermia is a condition where sperm cells have round heads without acrosomes. This often leads to failure in fertilisation because the sperm are unable to start the process of oocyte activation [32]. This condition poses a significant challenge, especially in the context of ICSI, as successful fertilisation

greatly depends on the sperm's ability to activate the oocyte. Although the evidence supporting the use of AOA in cases of globozoospermia is limited and of low quality, certain studies and clinical reports have demonstrated that AOA can enhance fertilisation rates in these individuals [30, 31]. Nevertheless, the recommendation lacks strength due to the low-quality evidence, indicating the necessity for cautious optimism. Given the insufficient reliability of the evidence, it is imperative for patients to be knowledgeable and actively engaged in the decision-making process, guaranteeing that their specific circumstances and preferences are thoroughly considered.

3. AOA for the general ICSI population

IVF facilities should NOT recommend AOA for the general ICSI population (very low-grade evidence—conditional recommendation against) [29, 33-37].

The recommendation against recommending AOA for the general ICSI population is justified by the limited and unreliable evidence currently accessible. Although AOA has demonstrated promising advantages in certain groups of patients, such as those with previous fertilisation failure [10, 11, 21, 24, 28], there is inadequate evidence to justify its regular application in all patients undergoing ICSI [37, 38]. The existing literature on AOA frequently has notable shortcomings, resulting in a dearth of strong and dependable data regarding the overall effectiveness and safety when used extensively in all ICSI patients. Standard protocols for most patients undergoing ICSI generally achieve satisfactory fertilisation rates without requiring additional interventions such as AOA. Implementing AOA universally may result in unnecessary intricacy, heightened expenses, and potential hazards without definitive proof of significant advantage [38]. For the broader ICSI population, the existing evidence base for AOA does not meet the required standard, suggesting avoiding AOA as a standard practice and instead only using it in specific cases where the potential benefits have been clearly proven.

4. AOA for improving embryo quality

IVF facilities should NOT recommend AOA for improving embryo quality (very low-grade evidence—conditional recommendation against) [13, 14, 39-41].

The recommendation against recommending AOA to enhance embryo quality in IVF facilities is grounded on the existing evidence, which is currently of insufficient quality [13, 14, 39-41]. The hypothesis that AOA could improve the quality of embryos has not been

adequately validated through rigorous scientific investigations. Embryo quality is a multifaceted result that is affected by various factors, such as the inherent quality of the egg and sperm, as well as the conditions in which the embryos are cultured [42]. The evidence indicating that AOA can directly improve the quality of embryos is limited, inconsistent, and plagued by methodological challenges. The precise biological mechanisms by which AOA may enhance embryo quality are not thoroughly comprehended, and current research does not establish a definitive connection between AOA and improved embryonic quality. The lack of comprehensive data weakens the rationale for utilising it in order to enhance the quality of embryos. When it comes to IVF, where the safety of patients and the effectiveness of treatment are of utmost importance, it is essential to rely on reliable evidence when making clinical decisions. The recommendation for using AOA to enhance embryo quality cannot be justified without sufficient empirical evidence. Instead, the emphasis should be on optimising well-established protocols that are supported by substantial evidence in order to enhance embryo quality. The recommendation to not use AOA for enhancing embryo quality is supported by the extremely poor quality of evidence and the absence of proven effectiveness.

5. AOA for improving embryo development and blastocyst formation

IVF facilities should NOT recommend AOA for improving embryo development and blastocyst formation (very low-grade evidence—conditional recommendation against) [13, 14, 39-41].

The recommendation against recommending AOA in IVF facilities for enhancing embryo development and blastocyst formation is supported by the limited and unreliable evidence currently accessible [13, 14, 39-41]. The processes of embryo development and blastocyst formation are affected by various factors, such as the genetic and epigenetic condition of the gametes, the culture environment, and the overall health of the oocyte [42]. Considering the limited and unreliable evidence, it is justified to discourage the routine use of AOA for enhancing embryo development and blastocyst formation. IVF facilities should avoid endorsing unproven methods for this purpose and instead focus on interventions supported by strong evidence to ensure the best possible outcomes.

6. Rescue AOA

IVF facilities should NOT recommend rescue AOA hours or day 1 after ICSI (very low-grade evidence—conditional recommendation against) [43-46].

Rescue AOA aims to initiate fertilisation later when the initial attempt to activate the oocyte after ICSI has failed to achieve timely fertilisation [47]. Nevertheless, the available evidence to substantiate the effectiveness and safety of this practice is severely restricted and of substandard quality. This could be due to limited sample sizes, the absence of control groups, and substantial methodological variability [43-46]. In addition, the timing of the rescue AOA procedure, which is performed several hours or on the first day after ICSI, introduces additional complexity and uncertainty. The postponed initiation may not sufficiently replicate the inherent physiological mechanisms necessary for optimal fertilisation and embryo growth. IVF facilities should abstain from adopting this rescue AOA practice and adhere to established techniques that guarantee the most favourable results for patients based on credible evidence.

7. AOA and sibling-oocyte-split

IVF facilities could consider sibling-oocyte-split if AOA is offered for patients of previous low or no fertilisation, given the scarce long-term safety data (good practice statment) .

The recommendation for IVF facilities to consider using a sibling-oocyte-split approach in cases where patients have experienced low or no fertilisation in the past is rooted in the principle of good practice. This is especially important considering the limited availability of long-term safety data for AOA [48]. The sibling-oocyte-split method entails the division of the inseminated oocytes into two distinct groups: one group undergoes AOA, while the other group does not [27, 37]. This methodology enables a straightforward evaluation of results within the identical treatment period. This approach enables the investigation of the potential advantages of AOA, especially for individuals who have had low or no fertilisation in previous cycles. At the same time, it reduces the risk of exposing all oocytes to an intervention with limited long-term safety information. This method offers the chance to transfer embryos from the group that underwent fertilisation without AOA if they are available. This can be particularly comforting for patients worried about AOA's implications on the future development of embryos and their children's health. By implementing a sibling-oocyte-split strategy, we can gather data to establish a more robust evidence base and evaluate whether AOA truly improves fertilisation rates while maintaining embryo quality. It is advisable to consider a sibling-oocyte-split if AOA is to be used for patients who have experienced low or no fertilisation in previous attempts. This recommendation is a valid and beneficial practice statment.

8. AOA for male factor infertility

Except for globozoospermia cases, IVF facilities should NOT recommend AOA for the ICSI cycle with male factor infertility and no history of no or low fertilisation (very low-grade evidence—conditional recommendation against) [9, 12, 29, 33, 34, 49-60].

The main reason for this recommendation is the insufficient evidence to support the effectiveness of AOA in enhancing fertilisation or clinical outcomes in this group of patients. AOA has demonstrated potential in particular situations, such as in patients with globozoospermia, where the structural abnormalities of the sperm directly hinder the natural activation of the oocyte [30, 31]. Nevertheless, there is limited evidence regarding the advantages of AOA for the overall male factor infertility population who have not experienced previous fertilisation failures. Male factor infertility encompasses a broad spectrum of problems, including reduced sperm count and motility, as well as abnormal sperm morphology. The underlying aetiology and mechanisms can exhibit significant variation, and there is a lack of conclusive evidence supporting the efficacy of AOA in addressing these multifaceted concerns [29, 33, 57]. The recommendation against AOA in this context takes into account the principle of avoiding interventions that lack robust evidence. The recommendation to avoid the use of AOA in ICSI cycles for male factor infertility, unless there is a history of no or low fertilisation, is supported by limited and unreliable evidence, as well as the lack of proven effectiveness.

9. General recommendation

Except for the above recommendations, AOA should only be considered within a research context without charging the patients and after proper counselling and written consent (good practice statment).

The recommendation that AOA should only be taken into account in a research setting, except for the specified situations, is grounded on sound principles. Although there are certain circumstances, such as globozoospermia or previous instances of low or no fertilisation, in which AOA may be advantageous, its wider utilisation lacks substantial evidence [38]. Therefore, it is advisable to limit the utilisation of AOA to a carefully regulated research context where its effectiveness and safety can be thoroughly assessed. Ensuring appropriate guidance and securing documented agreement are essential elements of this suggestion. Patients should receive comprehensive information regarding the experimental nature of AOA within a wider framework, the absence of definitive evidence

endorsing its efficacy, and the potential hazards and advantages involved. This level of transparency upholds the principle of patient autonomy and guarantees that patients are fully informed when making decisions about their involvement. In addition, performing AOA within a research framework allows for the monitoring and documenting long-term results, which helps to address existing gaps in the evidence. This data can enhance future guidelines and potentially result in more reliable recommendations if the effectiveness and safety of AOA are confirmed. This approach guarantees that the intervention is implemented with caution and ethical considerations.

Clinical indicators for monitoring:

We recommend strict follow-up of infertility units to ensure adherence to the recommendation of performing AOA and implementing an electronic database system to facilitate monitoring and adherence to the above recommendation. Practitioners should provide a clear justification and state the reason if they recommend AOA outside of the aforementioned recommendations.

- **Update of guideline**

  This guideline will be updated whenever new evidence is available.

- **Research gaps**

  Research in AOA reveals significant gaps that warrant further investigation. The field lacks high-quality evidence on AOA's effectiveness and safety across various patient groups, with a particular dearth of long-term safety data for children born after the procedure. There's limited understanding of AOA's impact on embryo quality and development, and the biological mechanisms underlying these effects remain unclear. The efficacy of rescue AOA and its optimal timing are poorly understood. The field would benefit from larger, well-designed studies with appropriate control groups to address these knowledge gaps.

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