

PREDICTIVE FACTORS FOR IMPROVED IN-VITRO-FERTILIZATION OUTCOMES WITH ARTIFICIAL OOCYTE ACTIVATION (AOA)

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INTRODUCTION

Failed fertilization remains a clinically significant challenge in assisted reproductive technology (ART), occurring in <3% of cycles (1). Failed fertilization after ICSI is frequently attributed to impaired oocyte activation, a calcium-dependent process initiated by sperm-derived phospholipase C zeta (PLCζ). Disruption of this pathway prevents the calcium oscillations required to trigger oocyte activation and subsequent embryogenesis (2).

Artificial oocyte activation (AOA) has emerged as a targeted intervention to overcome fertilization failure, particularly following low or failed fertilization with ICSI, by artificially inducing calcium oscillations to stimulate oocyte activation (3). Current European Society of Human Reproduction and Embryology (ESHRE) guidelines recommend consideration of AOA in cases of total fertilization failure, persistently low fertilization rates (<30%), or specific sperm-related defects such as globozoospermia (4).

Beyond its documented improvement in difficult fertilization ICSI cases, limited data evaluate the downstream effects of AOA on embryo development (5,6,7). Therefore, evidence remains scarce regarding its impact on embryo grading in larger cohorts or on blastocyst-stage outcomes, including aneuploidy rates assessed by PGT-A.

Despite a broadening application of AOA, including decreased fertilization rates and poor embryo development, standardized protocols and clear patient selection criteria for AOA are lacking. Given the additional gamete exposure associated with AOA, judicious identification of patients most likely to benefit is essential.

OBJECTIVE

To identify patients who most benefit from AOA through retrospective analysis. By characterizing IVF cycles of patients who previously had minimal or failed fertilization that were recommended AOA treatment, our goal is to generate clinically meaningful insights to inform patient counseling and support development of standardized AOA protocols.

METHODS

- Retrospective review of autologous IVF/ICSI cycles at a large academic-affiliated private center from August 2013 to August 2025
- Included all patients who underwent AOA/ICSI cycles with a previous ICSI only cycle
- AOA was performed by exposing injected oocytes to calcium ionophore (ionomycin in Hepes-buffered handling media) for four minutes, followed by a five-minute wash in high-protein media before standard culture.
- Variables: infertility diagnosis, sperm source, and prior IVF characteristics
- Primary outcomes were fertilization and usable blastocyst rates before and after AOA; secondary outcomes included pregnancy, live birth and PGT-A outcomes.
- Chi-squared tests and odds ratios (ORs) with 95% confidence intervals (CIs) were performed for statistical analysis.

Previous Cycle Fertilization Rate	Outcome	Previous Non-AOA Cycle (N = 116)	AOA Cycle (N = 103)	P-Value
0-25%	Fertilization Rate (2PN/MII injected)	173/1127 (15.4%)	456/958 (47.6%)	*p < 0.001
	Blast Rate (Blasts formed /2PN)	79/173 (45.7%)	258/456 (56.6%)	0.02
	Usable Blast Rate (Frozen + Transferred /2PN)	47/173 (27.2%)	136/456 (29.8%)	NS
	Freezable Blastocyst Rate (Frozen/2PN)	36/173 (20.8%)	112/456 (24.6%)	NS
	Total Blastocysts Frozen / Total Cycles	36/116 (0.31)	112/103 (1.09)	*p < 0.001
		Previous Non-AOA Cycle (N = 245)	AOA Cycle (N = 160)	
25-50%	Fertilization Rate (2PN/MII injected)	899/2332 (38.6%)	873/1548 (56.4%)	*p < 0.001
	Blast Rate (Blasts formed /2PN)	460/899 (51.2%)	477/873 (54.6%)	NS
	Usable Blast Rate (Frozen + Transferred /2PN)	236/899 (26.3%)	237/873 (27.1%)	NS
	Freezable Blastocyst Rate (Frozen /2PN)	187/899 (20.8%)	216/873 (24.7%)	NS
	Total Blastocysts Frozen / Total Cycles	187/245 (0.76)	216/160 (1.35)	NS
		Previous Non-AOA Cycle (N = 137)	AOA Cycle (N = 87)	
>50%	Fertilization Rate (2PN/MII injected)	921/1378 (66.8%)	546/860 (63.5%)	NS
	Blast Rate (Blasts formed /2PN)	549/921 (59.6%)	303/546 (55.5%)	NS
	Usable Blast Rate (Frozen + Transferred /2PN)	256/921 (27.8%)	144/546 (26.4%)	NS
	Freezable Blastocyst Rate (Frozen /2PN)	232/921 (25.2%)	128/546 (23.4%)	NS
	Total Blastocysts Frozen / Total Cycles	232/137 (1.69)	128/87 (1.47)	NS

TABLE 1.

IVF results before and after AOA/ICSI for autologous cycles with partner's fresh sperm

RESULTS

A total of 514 patients underwent 1,357 IVF cycles, of which 742 utilized AOA with ICSI. Among all AOA/ICSI cycles, 611 embryos were transferred, yielding an overall clinical pregnancy rate of 62.2%. The mean patient age was 37 years. Data are summarized in Table 1. Briefly, in autologous cycles the average fertilization rates were significantly higher with AOA compared to prior cycles with <50% fertilization rates. Among patients with <25% fertilization in previous cycles, AOA cycles resulted in a mean of 1.09 frozen blastocysts per cycle versus 0.31 in previous cycles (unadjusted rate ratio of 3.5: 95% CI 2.41, 5.10). AOA cycles produced 3.5 times more blastocysts per cycle than previous cycles. Patients with 0% prior fertilization achieved 42% fertilization with AOA.

The average proportion of good, fair, and poor-quality embryos was assessed per cycle. Embryo quality did not significantly improve after the application of AOA (Fig 1,2). Additionally, PGT-A outcomes on tested embryos showed a higher yield of euploid embryos in the subsequent AOA cycles. Notably, in patients <35 years the euploid rate changed from 45.51% to 66.15% (p = 0.004).

In both groups, frozen embryo transfer outcomes were assessed as shown in Table 2. Live birth was slightly higher in both the PGT-A tested and untested embryos after AOA, however not statistically significant. A total of 63/196 AOA/ICSI transfers resulted in a live birth compared to 38/197 ICSI only transfers resulted in live birth. All other pregnancy outcomes were similar between the two groups.

CONCLUSIONS

- AOA significantly improved fertilization rates in patients with prior fertilization cycles of ≤50%
- AOA increased the number of usable blastocysts per cycle
- Blastocyst development did not improve in any patient cohorts

This study is among the first to evaluate embryo quality and PGT-A outcomes at scale in AOA cycles. The observed difference in euploid rates among patients under 35 years was confirmed at both the embryo and paired patient levels, strengthening the robustness of our findings. Prospective studies are needed to further clarify the impact of AOA on embryo competence and clinical outcomes.

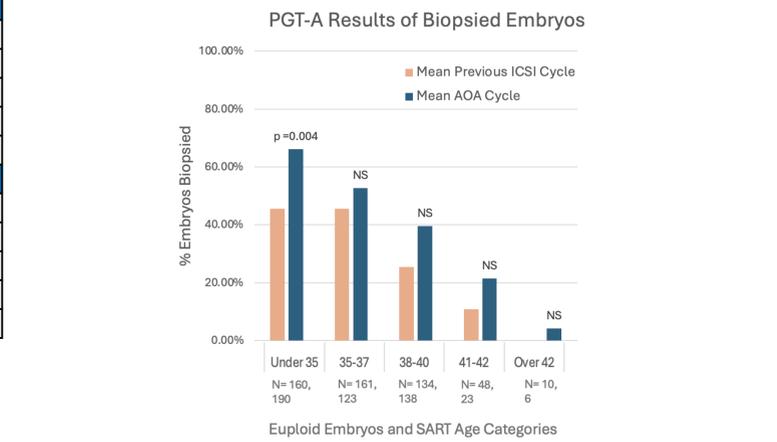
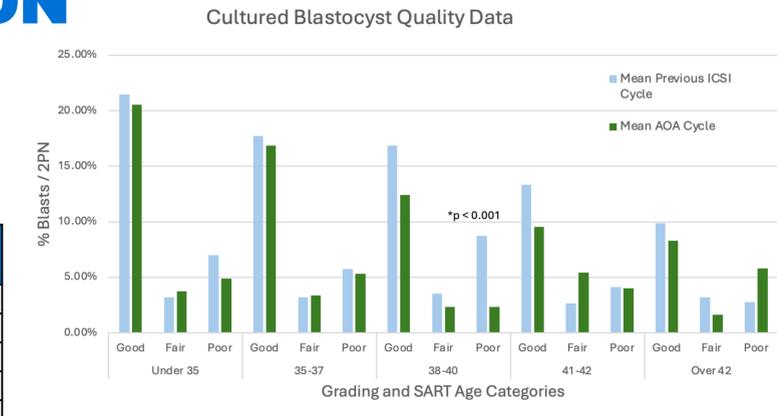


FIGURE 1,2. Embryo quality and PGT-A data of AOA/ICSI cycles

Non-PGT-A Transfers			
Outcomes	ICSI Only (N= 80)	AOA + ICSI (N= 68)	P-value
Biochemical Pregnancy	11.3%	4.4%	0.22
Clinical Pregnancy	40.0%	55.9%	0.08
Live Birth	17.5%	35.3%	0.02
Miscarriage	16.3%	8.8%	0.27
PGT-A Transfers			
Outcomes	ICSI Only (N= 117)	AOA + ICSI (N= 128)	P-value
Biochemical Pregnancy	15.4%	12.5%	0.64
Clinical Pregnancy	41.9%	46.9%	0.51
Live Birth	20.5%	30.5%	0.1
Miscarriage	10.3%	6.3%	0.36

TABLE 2. Pregnancy outcomes of frozen transfers of AOA/ICSI cycles

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