

Assisted oocyte activation significantly increases fertilization and pregnancy outcome in patients with low and total failed fertilization after intracytoplasmic sperm injection: a 17-year retrospective study

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Objective: To investigate the extent to which assisted oocyte activation (AOA) improves clinical outcomes in patients diagnosed with oocyte activation deficiencies (OADs).

Design: Retrospective cohort study comparing AOA cycles and previous intracytoplasmic sperm injection (ICSI) cycles in couples experiencing low or total failed fertilization after ICSI. Importantly, the sperm-related oocyte-activating capacity was examined in all patients before AOA with the use of the mouse oocyte activation test (MOAT).

Setting: Infertility center at a university hospital.

Patient(s): A total of 122 couples with a history of low or total failed fertilization after ICSI.

Intervention(s): ICSI, MOAT, AOA, and embryo transfer.

Main Outcome Measure(s): Fertilization, pregnancy, and live birth rates.

Result(s): MOAT revealed 19 patients with a sperm-related OAD (MOAT group 1), 56 patients with a diminished sperm-related oocyte-activating capacity (MOAT group 2), and 47 patients with a suspected oocyte-related OAD (MOAT group 3). AOA (191 cycles) significantly improved fertilization, pregnancy, and live birth rates in all MOAT groups compared with previous ICSI attempts (243 cycles). Fertilization rates after AOA were significantly different among MOAT groups 1 (70.1%), 2 (63.0%), and 3 (57.3%). Between MOAT group 1 and 3, significant differences in pregnancy (49.0% vs. 29.4%) and live birth (41.2% vs. 22.1%) rates were observed. In total, 225 embryo transfers resulted in 60 healthy live births following AOA.

Conclusion(s): Patients undergoing diagnostic testing before AOA show a significant improvement in clinical outcomes compared with previous cycles. Our findings highlight that AOA should be reserved for patients with clear OADs. (*Fertil Steril*® 2019;112:266–74. ©2019 by American Society for Reproductive Medicine.)

El resumen está disponible en Español al final del artículo.

Key Words: Intracytoplasmic sperm injection, fertilization failure, mouse oocyte activation test, calcium ionophore, assisted oocyte activation

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Intracytoplasmic sperm injection (ICSI) has become the most popular assisted reproductive technique applied for several infertility indications (1). Although normal fertilization rates are estimated at ~70%, total fertilization failure (TFF) still occurs in 3%–5% of all ICSI cycles (2). The main cause for TFF after ICSI has been attributed to oocyte activation deficiencies (OADs), which can be related to sperm or oocyte factors (3, 4).

Oocyte activation is a universal process comprising a complex series of molecular events that are essential for fertilization (5). The initiation of the oocyte activation process is triggered by a sperm oocyte-activating factor, phospholipase C zeta (PLC ζ), which is delivered into the oocyte at sperm-egg fusion (6). In mammals, a typical pattern of multiple calcium (Ca²⁺) increases, namely Ca²⁺ oscillations, is observed after the fusion of the sperm and the oocyte. These Ca²⁺ oscillations are required to achieve the resumption of the meiotic cell cycle and ultimately successful fertilization (3). Both the sperm and the oocyte play important roles: the sperm by supplying its functional sperm factor PLC ζ and the mature oocyte by being receptive to PLC ζ and generating the correct Ca²⁺ pattern.

The use of ICSI in combination with assisted oocyte activation (AOA) has gained popularity over the past two decades as a treatment for patients experiencing fertilization failure after ICSI (3, 4, 7, 8). AOA aims to provide a sufficient intracellular Ca²⁺ release, allowing successful oocyte activation and fertilization (4, 9). The AOA technique is a procedure by which Ca²⁺ rises are artificially induced in the oocyte, with Ca²⁺ ionophores being the most commonly applied technique (3). However, there is currently no clinical consensus regarding the appropriate AOA treatment protocol. Moreover, patients are commonly treated without a clear indication. For example, Borges et al. (2009) performed AOA in the first ICSI cycle for abnormalities in sperm morphology without prior diagnosis of the cause of failed fertilization (10). As such, clinics do not achieve consistent outcomes (i.e., extensive variability in fertilization rates) and often do not observe a direct benefit of AOA (11). Moreover, the use of particular artificial activation agents remains controversial. We have previously shown that calcimycin (GM508 Cult-Active), used by many IVF laboratories to overcome fertilization failure, is significantly less efficient compared with ionomycin-based AOA (12).

Two decades ago, we developed a diagnostic method to evaluate the oocyte-activating capacity of human spermatozoa (8, 13): the mouse oocyte activation test (MOAT), which has also been performed by other centers (14–16). In accordance with the MOAT result, patients are diagnosed with sperm-related OAD (MOAT group 1), diminished oocyte-activating capacity of the sperm (MOAT group 2), or suspected oocyte-related OAD (MOAT group 3) (8). Importantly, MOAT serves as a valuable tool for appropriate patient counseling, particularly regarding gamete donation. Moreover, because studies have shown that OADs may have an underlying genetic cause, such as mutations in the PLC ζ gene (17), patients can be further informed about risks of OAD transmission to children born after AOA.

To date, large-scale studies assessing the efficiency of AOA in patients experiencing TFF or low fertilization (LF; <33%) after ICSI with prior diagnostic testing for an OAD are lacking. Here, we performed a retrospective cohort study, including patients with previous TFF or LF after ICSI, for whom the sperm-related oocyte-activating capacity was examined by MOAT before AOA treatment. Moreover, we investigated to what extent AOA improved clinical outcomes in patients diagnosed with OADs.

MATERIALS AND METHODS

Study Design, Setting, and Participants

A retrospective cohort study was performed, including 122 couples with a history of TFF (fertilization rate of 0%–10%) or LF (fertilization rate of \leq 33.3%, based on the 5th percentile of mean fertilization rates) after conventional ICSI. The sperm-related oocyte-activating capacity of all patients was examined by means of MOAT, performed from January 2001 to December 2017 at the research facility at Ghent University Hospital. All patients underwent AOA from April 2001 to April 2018 at the IVF facility at Ghent University Hospital.

Sperm Preparation

Both frozen-thawed and fresh human ejaculated sperm samples were washed by means of centrifugation (1600 rpm, 10 minutes) with Sydney IVF Gamete Buffer (Cook). For clinical cycles (ICSI or ICSI-AOA), standard sperm selection was performed with the use of density-gradient centrifugation, whereas for MOAT, the swim-out sperm selection procedure was carried out for all sperm samples unless a very low sperm count was observed. Only motile sperm were recovered and mechanically immobilized before ICSI.

MOAT Procedure

MOAT was performed on all patients as previously described (18). Briefly, mature mouse oocytes were collected from superovulated B6D2F1 hybrid female mice (Janvier Labs) and cultured in KSOM media.

Four different groups were required to execute a complete test: 1) injection of at least 40 oocytes with patient spermatozoa; 2) injection of at least 40 oocytes with control (research-donated) spermatozoa with proven fertilization capacity (positive control); 3) sham injection of at least 10 oocytes (medium injection without spermatozoa; negative control); and 4) at least 10 medium control oocytes (nonmanipulated mouse oocytes; negative control). Injections were performed at 5–10 °C in HEPES-buffered KSOM, 20% fetal bovine serum (Gibco) with the use of piezoelectric pulses.

After micromanipulations, oocytes were cultured in KSOM and checked for two-cell formation (activation rate) 24 hours later. Depending on the activation rate, patients were classified into MOAT group 1 (\leq 20% activation, i.e., the upper limit of the negative control), MOAT group 2 (21%–84% activation), or MOAT group 3 (\geq 85% activation, i.e., the lower limit of the positive control) (8). All cultures were performed at 37 °C in 6% CO₂, 5% O₂, and 89% N₂.

Ovarian Stimulation

Many patients undergoing MOAT and AOA are referred to our center from other IVF clinics (75%) where they experienced TFF or LF after ICSI. As such, we do not possess all the information regarding the stimulation protocols of their previous ICSI cycles. In the majority of the AOA cycles, a short agonist treatment was chosen for controlled ovarian stimulation; however, in 5% and 16% of the AOA cycles, a long agonist or antagonist protocol was chosen, respectively. Ovulation was triggered by administering hCG. We delayed our interval of denudation because all AOA pick-ups were planned as early as possible and denudation only occurred just before the AOA.

AOA Procedure

Thirty-six hours after hCG administration, oocytes were retrieved by means of transvaginal ultrasound-guided puncture of the ovarian follicles in HTF-HEPES medium (Origio). Cumulus-oocyte complexes were briefly exposed to hyaluronidase (Cumulase; Origio) followed by mechanical denudation. Subsequently, oocytes were cultured in Sydney IVF Fertilization Medium (Cook).

The AOA procedure was performed as previously described (8). Briefly, freshly collected mature oocytes were injected with patients' spermatozoa along with 0.1 mol/L CaCl₂ (Sigma-Aldrich), corresponding to the diameter of the oocyte (19). After a 30-minute incubation period, oocytes underwent a twofold exposure to ionomycin (10 μmol/L, I9657; Sigma-Aldrich) for 10 minutes with a 30-minute interval before culture. Embryos were cultured in Sydney IVF Cleavage Medium (Cook) until day 3 (D3) and in Sydney IVF Blastocyst Medium (Cook) from D3 to D5.

Embryo Grading

Embryos were classified into four quality categories in accordance with European Society for Human Reproduction and Embryology guidelines (20) (Supplemental Table 1; Supplemental Tables 1–5 are available online at www.fertstert.org).

Embryo Transfer

Embryo transfers (ETs) were performed on D2, D3, or D5. Criteria for ETs changed during the study period. Before 2012, ETs were predominately performed on D2 or D3. In 2012 and 2013, ETs were performed on D5 when at least ten diploid zygotes were obtained, otherwise on D3. In 2014 and 2015, the number of diploid zygotes required for a D5 ET was reduced to five. From 2016 on, only D5 ETs were performed, regardless of the number of zygotes.

Study Outcomes

In accordance with the MOAT result, AOA efficiency was determined based on fertilization, pregnancy, and live birth rates compared with previous conventional ICSI cycle outcomes. Information regarding previous ICSI cycles was obtained from the IVF facility at Ghent University Hospital

and other IVF centers both internationally and within Belgium.

Statistics

The chi-square test, Kruskal-Wallis test, and Mann-Whitney *U* test were used for statistical analysis with the use of the Statistical Package for Social Sciences (SPSS Statistics 25; IBM), as appropriate. *P* values < .05 were considered to be statistically significant.

Study Approval

Written informed consent for the MOAT and AOA treatment was obtained from every couple (B670201423110). The use of laboratory animals for performing the MOAT was approved by the Ghent University Hospital Ethical Committee for Laboratory Animals (ECD no. 15/55).

RESULTS

Participants

In total, 122 couples with a history of TFF or LF after ICSI (243 ICSI cycles) were included in the study. A mean of 7.8 oocytes per cycle were injected with motile sperm in previous ICSI attempts, with an unknown underlying cause of fertilization failure (21, 22). The mean maternal ages among the different MOAT groups were 31.2 years, 33.9 years, and 33.7 years for MOAT groups 1, 2, and 3, respectively. All couples underwent conventional ICSI, MOAT, and AOA, except for seven patients in MOAT group 1 who did not undergo conventional ICSI after the diagnosis of globozoospermia. The mean time between the MOAT and subsequent AOA treatment was 13.4 ± 9.4 weeks and that between ICSI and AOA, 39.7 ± 27.0 weeks.

MOAT Distribution

The MOAT revealed 19 patients with a sperm-related OAD (MOAT group 1), 56 patients with a diminished oocyte-activating capacity of the sperm (MOAT group 2), and 47 patients with a presumably oocyte-related OAD (MOAT group 3). Except for one patient with another type of teratozoospermia, all MOAT group 1 patients were diagnosed by means of routine semen analysis with partial (*n* = 1) or total (*n* = 17) globozoospermia. Semen parameters for all MOAT groups are presented in Supplemental Table 2.

AOA Outcome

In total, 191 AOA cycles were performed at our center from April 2001 to April 2018. The overall fertilization rate after AOA treatment was 63.3% (1,104/1,743), which was significantly higher than previous ICSI attempts, 15.2% (286/1,884; *P* < .001). Fertilization rates increased significantly from 9.7%, 14.8%, and 17.7% after conventional ICSI to 70.1%, 63.0%, and 57.3% after AOA in MOAT groups 1, 2, and 3, respectively (*P* < .001; Table 1). After AOA treatment, MOAT group 1 patients showed significantly higher fertilization rates compared with MOAT group 2 patients (*P* = .01), which had a significantly higher fertilization rate than

TABLE 1

Comparison of preimplantation characteristics and embryo transfer outcomes after previous ICSI and subsequent AOA treatment within and between the different MOAT groups, comprising patients with previous low or total failed fertilization after conventional ICSI.

Parameter	MOAT group 1 (n = 19)		MOAT group 2 (n = 56)		MOAT group 3 (n = 47)	
	ICSI (n = 20)	AOA (n = 35)	ICSI (n = 140)	AOA (n = 89)	ICSI (n = 83)	AOA (n = 67)
Treatment (oocyte retrievals)						
Cumulus-oocyte complexes	246	572	1414	1129	816	650
Fertilization rate (2PN/MII)	9.7% (18/185) ^a	70.1% (330/471) ^b	14.8% (163/1,104) ^{a,c}	63.0% (500/794) ^d	17.7% (105/595) ^c	57.3% (274/478) ^e
Fresh ET	9	34	68	65	40	53
Mean fresh embryos transferred	0.6 ± 0.8	1.5 ± 0.7	0.6 ± 0.7	1.1 ± 0.9	0.6 ± 0.7	1.2 ± 0.8
Embryos cryopreserved	2	113	11	132	6	59
Frozen ET	0	17	3	41	3	15
Mean frozen embryos transferred	0.0 ± 0.0	0.6 ± 0.9	0.0 ± 0.1	0.5 ± 1.0	0.0 ± 0.3	0.3 ± 0.8
Retrievals with fresh/frozen ET	45.00% (9/20) ^a	100.00% (35/35) ^b	49.29% (69/140) ^a	75.28% (67/89) ^c	48.19% (40/83) ^a	80.60% (54/67) ^c
Pregnancy rate (hCG+/ET)	0.0% (0/9) ^a	49.0% (25/51) ^b	7.0% (5/71) ^a	34.9% (37/106) ^{b,c}	7.0% (3/43) ^a	29.4% (20/68) ^c
Fresh cycle	0.0% (0/9)	50.0% (17/34)	7.4% (5/68)	29.2% (19/65)	7.5% (3/40)	28.3% (15/53)
Thaw cycle	0.0% (0/0)	47.1% (8/17)	0.0% (0/3)	43.9% (18/41)	0.0% (0/3)	33.3% (5/15)
Biochemical pregnancy	0	0	2	6	2	3
Ectopic pregnancy	0	0	0	0	0	1
Mors in utero	0	0	0	0	1	0
Miscarriage	0	4	1	9	0	1
Ongoing pregnancy (>12 wk)						
Fresh cycle	0	14	2	12	0	12
Thaw cycle	0	7	0	10	0	3
Live birth rate (live births/ET)	0.0% (0/9) ^a	41.2% (21/51) ^b	2.8% (2/71) ^a	22.6% (24/106) ^c	0.0% (0/43) ^a	22.1% (15/68) ^c
Fresh cycle	0	14	2	15	0	12
Thaw cycle	0	7	0	9	0	3

Note: Pregnancy rate was defined as the proportion of embryo transfers resulting in a positive hCG test (>10 mIU/mL). 2PN = two pronuclei; AOA = assisted oocyte activation; ET = embryo transfer; ICSI = intracytoplasmic sperm injection; MII = metaphase II; MOAT = mouse oocyte activation test.

^{a,b,c} Different superscript letters indicate statistical significance between groups ($P < .05$).

Bonte. AOA overcomes failed fertilization. *Fertil Steril* 2019.

MOAT group 3 patients ($P = .04$; Table 1). When evaluating patients experiencing TFF and LF after ICSI separately, we observed that fertilization rates after AOA were almost identical in both patient subgroups, regardless of the MOAT group ($P > .05$; Supplemental Table 3).

Overall, one patient in MOAT group 1 and one in MOAT group 2 with histories of TFF after ICSI still experienced TFF after AOA treatment. In MOAT group 3, AOA did not result in any successful oocyte activation in three out of 26 patients with previous LF and two out of 21 patients with previous TFF after ICSI (Fig. 1). Furthermore, nine MOAT group 2 patients, five with previous LF and four with previous TFF after ICSI, experienced LF after the application of AOA. Also, eight MOAT group 3 patients, five with previous TFF and three with LF after ICSI, experienced LF after AOA (Fig. 1).

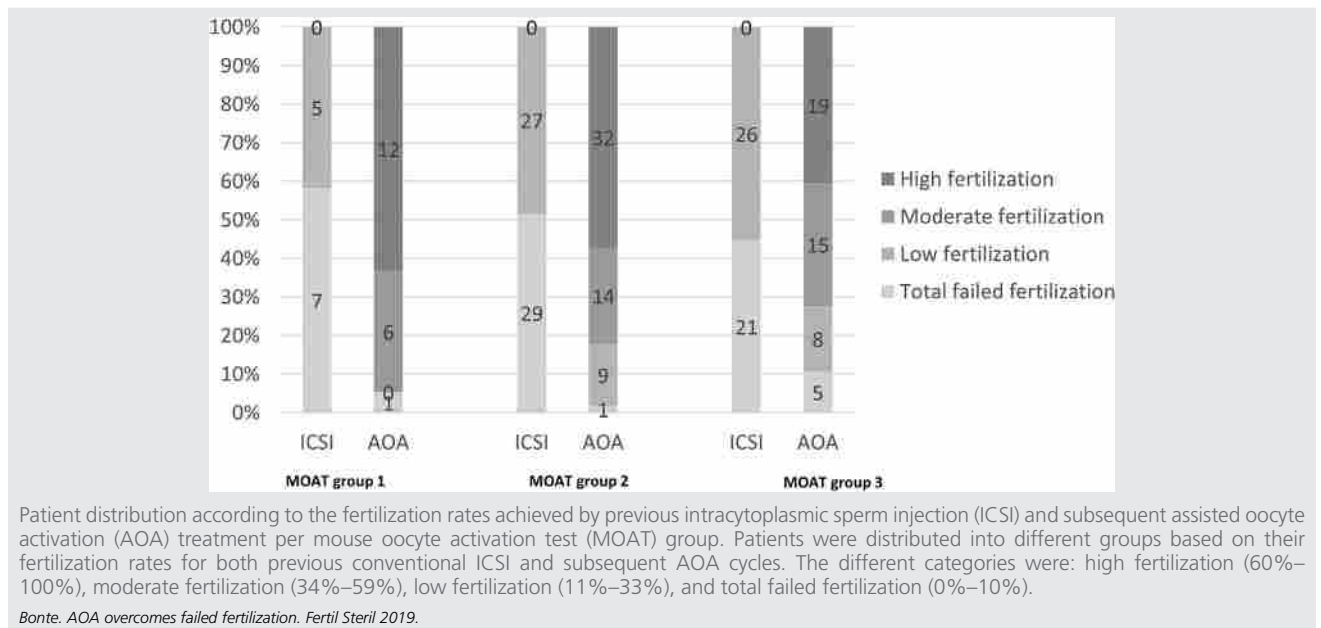
Although outcomes were variable between different ET policies, some significant differences were observed in terms of embryo quality (categories A and B) among the different MOAT groups (Fig. 2). Overall, blastocyst rates after AOA were within the normal range in all MOAT groups (Fig. 2; Supplementary Table 4). We report on embryo scores for

AOA cycles only, because information regarding embryo quality from previous ICSI cycles was not provided by most previous centers.

Overall, all 19 MOAT group 1 patients, 44 of the 56 MOAT group 2 patients, and 37 of the 47 MOAT group 3 patients had a fresh or frozen ET. Pregnancy rates were significantly higher after AOA (36.4%, 82/225) compared with previous conventional ICSI (6.5%, 8/123; $P < .001$), increasing significantly from 0.0%, 7.0%, and 7.0% after conventional ICSI to 49.0%, 34.9%, and 29.4% after AOA in MOAT groups 1 ($P = .006$), 2 ($P < .001$), and 3 ($P = .005$), respectively (Table 1). When evaluating D2/D3 and D5 ETs separately, pregnancy rates still increased after AOA compared with previous ICSI cycles for both groups (Supplemental Table 5). In total, 14 (17.1%) spontaneous abortions, 9 (11.0%) biochemical pregnancies, and 1 (1.2%) ectopic pregnancy occurred.

Live birth rates were significantly higher after AOA (26.7%, 60/225) compared with previous conventional ICSI (1.6%, 2/123; $P < .001$), increasing significantly from 0.0%, 2.8%, and 0.0% after conventional ICSI to 41.2%, 22.6%, and 22.1% after AOA in MOAT groups 1 ($P = .02$), 2

FIGURE 1



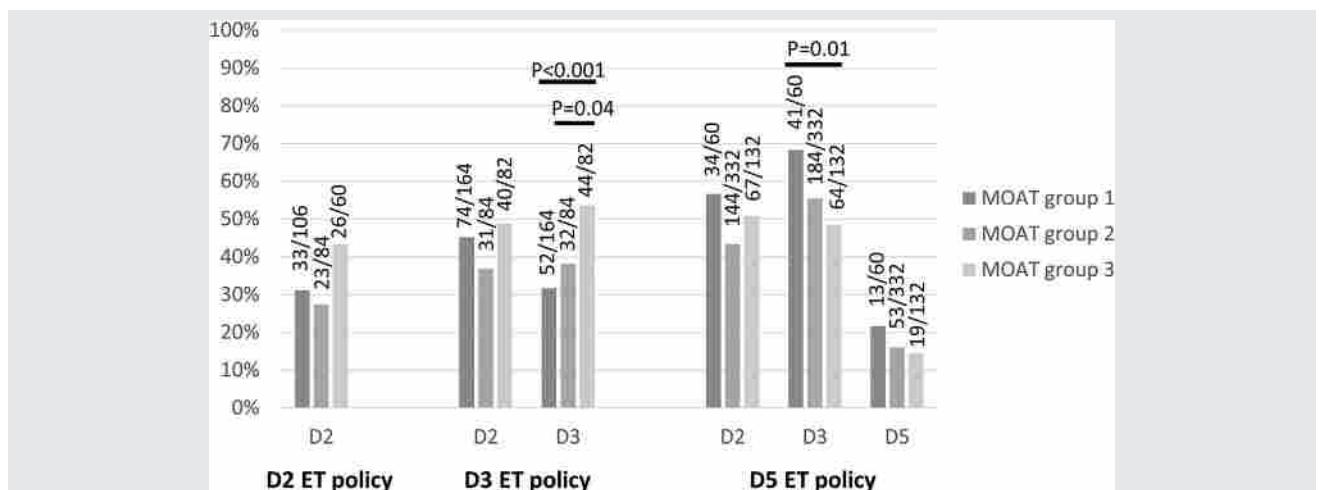
($P < .001$), and 3 ($P < .001$), respectively (Table 1). When evaluating D2/D3 and D5 ETs separately, live birth rates also significantly increased after AOA compared with previous ICSI cycles for both groups (Supplemental Table 5). In total, 21, 21, and 14 clinical pregnancies developed to term in MOAT groups 1, 2, and 3, respectively (Table 2). Finally, 52 singletons and 4 twins (three twins in MOAT group 2 and one twin in MOAT group 3) were born. None of the 32 boys and 28 girls had major or minor congenital malformations. Only two pregnancies ended up in preterm deliveries: twins born at 36 weeks 4 days and twins born at 36 weeks, of which

one twin baby had low weight (2,180 g). Another twin baby (born at 38 weeks 5 days) also had a slightly lower weight (2,480 g) (Table 2). Delivery type, birth weight, and Apgar scores of, respectively, 7, 6, and 13 babies were missing. Two clinical pregnancies were still ongoing.

DISCUSSION

This is the first study to describe the efficiency of AOA in a large series of patients with a history of TFF or LF (<33%) after ICSI, for whom the oocyte-activating capacity of the

FIGURE 2



Percentages of two-pronuclei (2PN) zygotes that developed into excellent-quality (A) and good-quality (B) embryos after AOA among the different MOAT patients and categorized by the different embryo transfer (ET) policies. Every ET policy is subdivided into A+B embryo quality and grading timepoint (day 2, 3, and 5). Fractions above the bars indicate the proportion of 2PNs that developed to A+B quality embryos. D2 = day 2; D3 = day 3; D5 = day 5; other abbreviations as in Figure 1.

TABLE 2

Obstetrical and neonatal outcomes of babies born after assisted oocyte activation.

Outcome	Singletons (n = 52)	Twins (n = 4)	Total (n = 60)
Obstetrical outcomes			
Preterm delivery (<37 wk)	0	4	4
Vaginal delivery	30	0	30
Cesarean section	15	8	23
Neonatal outcomes			
Mean birth weight (g)	3,556 ± 545	2,825 ± 429	3,435 ± 587
Birth weight <2,500 g	0	2	2
Apgar score <7 at 5 min	0	0	0
Perinatal mortality	0	0	0
Sex			
Male	27	5	32
Female	25	3	28

Delivery type, birth weight, and Apgar scores of respectively 7, 6, and 13 babies are missing. Bonte. AOA overcomes failed fertilization. *Fertil Steril* 2019.

sperm was examined by means of MOAT. Our data demonstrate that AOA with the use of CaCl_2 injection in combination with a twofold ionomycin exposure resulted in significantly higher fertilization, pregnancy, and live birth rates in all MOAT groups compared with previous ICSI cycles. Patients with TFF after ICSI had similar outcomes with AOA compared with patients with LF after ICSI (Supplemental Table 3). These results confirm earlier findings reported by our group, describing an improvement in fertilization rates in all three MOAT groups in a small series of patients (30 couples, 61 AOA cycles) (8). Moreover, blastocyst rates were within the normal range in all MOAT groups. Although some patients underwent multiple AOA cycles, no differences were seen in AOA outcomes between the first AOA cycle for each patient and all AOA cycles combined.

Except for one globozoospermic patient who still experienced TFF after AOA, the other 17 patients with globozoospermia showed fertilization rates up to 90% (range 35.0%–89.7%) and pregnancy rates of 49.0%. After AOA, a high number of two-pronuclei zygotes developed into excellent- and good-quality embryos. Overall, patients diagnosed with a sperm-related OAD responded significantly better to the AOA treatment in terms of fertilization, pregnancy, and live birth rates compared with patients with a suspected oocyte-related OAD.

We report some early spontaneous abortions, but the frequencies were similar to those in other AOA studies (23, 24). Overall, our findings show that AOA treatment was a safe and effective technique resulting in the birth of 60 healthy babies, confirming earlier safety reports (24–26). However, continuous follow-up of children born after AOA is warranted.

Four MOAT 3 patients opted to participate in an oocyte donation program instead of AOA. Fertilization rates increased significantly from 31.0% (n = 8 cycles; own oocytes) to 75.0% (n = 8 cycles; donor oocytes; $P < .001$)

and resulted in the birth of two healthy singletons. These outcomes further reinforce the importance of performing a diagnostic test in patients with fertilization failure after ICSI for appropriate counseling (8, 18, 27), still lacking in many centers worldwide. By performing MOAT we are able to: 1) better predict the success of AOA; 2) provide improved clinical management, especially regarding gamete donation in cases when AOA could not overcome fertilization failure; and 3) diagnose patients with mutations in oocyte activation-related genes, such as the sperm factor PLC ζ (especially in MOAT group 1 and 2 patients), more readily (17). This is particularly valuable for making patients aware of the possible risk of transmitting genetic subfertility to their offspring after AOA. Research has shown that genetic mutations in PLC ζ have been associated with OAD and fertilization failure. To date, four missense mutations in the PLC ζ gene have been discovered in four different patients experiencing TFF or LF after ICSI, including H398P, H233L, I489F, and R197H (28–31). Moreover, mutations in oocyte-related factors, such as inositol 1,4,5-trisphosphate receptor (IP3R), Ca^{2+} /calmodulin-dependent protein kinase II, protein kinase C, and stromal interaction molecule-1, may also contribute to OAD, especially in cases where an oocyte-related OAD is suspected (i.e., MOAT group 3) (32). Nevertheless, further research is required to elucidate the full spectrum of female- and male-related genes essential for successful fertilization.

Ca^{2+} ionophore treatment is capable of overcoming not only sperm-related OADs, but also some suspected oocyte-related OADs diagnosed by means of MOAT (18). However, human PLC ζ has a greater potency to activate mouse oocytes compared with mouse PLC ζ (33). Studies have shown that the sperm of some MOAT group 3 patients can still induce aberrant Ca^{2+} oscillatory patterns in some instances (7, 34). However, most MOAT 3 patients may have an oocyte-related OAD due to a defect in the Ca^{2+} -releasing machinery of the oocyte, such as IP3R. With the use of MOAT, however, we investigate only the spermatozoa for their oocyte-activating capacity. We therefore cannot exclude the possibility that MOAT group 1 and 2 patients may have an OAD contributed by both partners. In most cases, this oocyte-related OAD will also be overcome by AOA (18). Nevertheless, patients with oocyte-related OADs (i.e., MOAT group 3) may still experience TFF or LF after AOA, which could be attributed to several other factors. These may include poor overall oocyte quality, nuclear defects, defects in the cytoskeleton associated with store-operated Ca^{2+} entry, or defects downstream of the intracellular Ca^{2+} -releasing process (18, 32). Developing further assays to diagnose oocyte-related factors relating to fertilization will undoubtedly provide improved clinical management and treatment (32).

The inclusion of a large cohort of patients forms the strength of this study. However, ICSI cycle data were obtained from both in-house (25%) and other IVF laboratories (75%). Therefore, some data remain unknown (e.g., initial indication for ICSI), and differences in clinical practices, such as ovarian stimulation protocols, may introduce variability. Because these data are not readily available from other IVF centers, we cannot exclude the possibility that some patients may have benefited from the use of a different stimulation regimen

during their AOA cycle, especially in cases where an oocyte-related OAD is suspected (i.e., MOAT group 3). However, several AOA studies to date face similar potential bias (35–37). Nevertheless, similar ICSI fertilization rates were observed in both in-house (11.4%, 32/281) and outside (15.8%, 254/1,603) cycles ($P=.06$). Although differences in ET policies may introduce variability, our findings demonstrate that pregnancy and live birth rates were higher after AOA compared with previous ICSI, regardless of whether ET was performed on D2/D3 or D5 (Supplemental Table 5).

To date, Ca^{2+} ionophores such as ionomycin and calcimycin are the most commonly used chemical activating agents to overcome fertilization failure after ICSI (3, 4). In patients with TFF or LF after ICSI, fertilization rates with the use of calcimycin were much lower (40%–50%) than those achieved after AOA with the use of ionomycin (up to 70%) (35, 38). Those findings confirmed our previous results in mice and humans establishing ionomycin-based oocyte activation as more efficient (12). Some studies have reported successful fertilization resulting in healthy live births by means of strontium chloride ($SrCl_2$) activation in couples with previous fertilization failure (39, 40). However, its use and mechanism of action are still controversial, because Ca^{2+} oscillations are not observed in human oocytes after direct exposure to $SrCl_2$ (41, 42). Variability between AOA protocols and thus AOA outcomes complicates comparisons of efficiency (4). Specifically, Lu et al. (2018) show that differences in both ionomycin and Ca^{2+} concentration in culture media during AOA can have a negative effect on the efficiency in both mice and humans (42).

Despite the added value of MOAT, more sensitive diagnostic tests, such as Ca^{2+} pattern analysis of the patient's spermatozoa in mouse (mouse oocyte calcium analysis; MOCA) or human oocytes (human oocyte calcium analysis; HOCA) would aid in predicting the success of AOA and guiding further clinical management of fertilization failure after ICSI (7, 34). Still, these novel diagnostic tests require specialized equipment and infrastructure, as well as the use of human oocytes for research, which limits their application.

Because AOA is still an experimental procedure, our findings highlight that its use should be reserved for a specific subgroup of patients, i.e., those with clear OADs. Patients who underwent diagnostic testing of the oocyte-activating potential of spermatozoa before their AOA treatment show a significant improvement in fertilization, pregnancy, and live birth rates. Importantly, diagnosis is paramount for improved clinical management and diligent counseling of patients experiencing failed fertilization after ICSI.

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La activación asistida de los ovocitos aumenta de manera significativa la fecundación y los resultados gestacionales en pacientes con un fallo de fecundación bajo o total después de la microinyección espermática: un estudio retrospectivo de 17 años

Objetivo: Investigar en qué medida la activación asistida de ovocitos (AOA) mejora los resultados clínicos en pacientes diagnosticados de deficiencia de activación ovocitaria.

Diseño: Estudio retrospectivo de cohortes comparando AOA ciclos con ciclos previos con microinyección espermática en parejas que experimentaron bajo o total fallo de fecundación tras ICSI. Notablemente, la capacidad de activar el ovocito relacionado con el semen fue examinada en todos los pacientes antes AOA con el uso de un test de activación ovocitaria en ratones (MOAT).

Lugar: Centro de infertilidad en un hospital universitario.

Paciente (s): Un total de 122 parejas con historia de bajo o total fallo de fecundación tras ICSI.

Intervención (es): ICSI, MOAT, AOA y transferencia embrionaria.

Principales medidas de resultados: Fecundación, gestación, y tasas de recién nacidos vivos.

Resultados: MOAT reveló 19 pacientes con OAD (MOAT grupo 1) relacionado con el semen, 56 pacientes con una capacidad de activación ovocitaria relacionada con el semen (MOAT grupo 2), y 47 pacientes con una sospecha de OAD relacionada con el semen (MOAT grupo 3). AOA (191 ciclos) mejoró significativamente la fecundación, gestación y tasa de recién nacido vivo en todos los grupos de MOAT comparados con los intentos previos de ICSI (243 ciclos). Las tasas de fecundación después de AOA fueron significativamente diferentes entre grupo MOAT 1 (70.1%), 2 (63.0%), Y 3 (57.0%). Entre grupo 1 y 3 de MOAT se observaron diferencias significativas en gestación (49.0% vs 29.4%) y en tasa de recién nacido vivo (41.2% vs 22.1%). En total, de 225 transferencias embrionarias resultaron 60 niños sanos tras AOA.

Conclusión (es): Pacientes que llevaron a cabo test diagnósticos antes AOA muestran una mejora significativa en los resultados clínicos comparado con los ciclos previos. Nuestros hallazgos destacan que AOA debería ser reservado para pacientes con OADs claro.