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Artificial oocyte activation to improve reproductive outcomes in couples with various reproductive problems: a retrospective cohort study

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Abstract

Research question Does calcium ionophore (A23187) treatment of oocytes improve fertilization rate, embryo development, and outcomes in selected groups of infertile couples?

Design This was a retrospective cohort study involving 796 couples undergoing oocyte activation with calcium ionophore after ICSI between the years 2016 and 2018, All metaphase II oocytes of the participants were exposed to 5 μmol/L ionophore for 15 min immediately after ICSI, cultured *in vitro* to the blastocyst stage, and then transferred to the uteri of recipients at Day 5 or cryopreserved for transfer in the next cycle. The previous cycles of the same patients were treated as the control group.

Results On the basis of a cohort of 1261 ICSI cycles and 796 ICSI-artificial oocyte activation (ICSI-AOA) cycles, we found that the rates of implantation, positive β-hCG, clinical pregnancy, and live birth were significantly improved compared with the previous cycles. Additionally, compared with previous cycles, the rates of blastulation and high-quality blastocysts were increased significantly for couples with male factors, young patients with chronic salpingitis, and infertile couples with both factors in the ICSI-AOA cycles. The miscarriage rate was decreased significantly for the couples with male factors, young patients with chronic salpingitis, and patients with polycystic ovary syndrome (PCOS) in the treatment cycles. However, no

significant differences were found for fertilization rate, embryo development, or outcomes in patients with primary ovarian insufficiency between the ICSI and ICSI-AOA groups.

Conclusions AOA was able to "rescue" the poor reproductive outcomes in certain types of infertile couples with history of failure pregnancy.

Key words: calcium ionophore, artificial oocyte activation (AOA), ICSI, pregnancy, live birth

Introduction

Intracytoplasmic sperm injection (ICSI), a well-known technique in which a single sperm is microinjected directly into the mature oocyte, is predominantly used to treat severe male factor infertility for achieving fertilization and previously failed *in vitro* fertilization (IVF) cycles (Palermo *et al.*, 1992). The fertilization rate of ICSI is about 70%, and complete or virtually complete fertilization failure still occurs at an estimated rate of 1% to 5% of all ICSI cycles and can recur in repeated cycles (Kashir *et al.*, 2010; Miller *et al.*, 2016). Failed fertilization after ICSI may be caused by failure of oocyte activation or an abnormality of the oocyte. Previous reports have shown that failure of oocyte activation is the major reason for fertilization failure after ICSI (Dozortsev *et al.*, 1994; Tesarik *et al.*, 1994; Lu *et al.*, 2006; Nasr-Esfahani *et al.*, 2007).

Oocyte activation is a complex spatiotemporal process in most mammals, triggered by sperm entry, which results in oscillations of intracellular calcium released from endoplasmic reticulum stores (Tesarik *et al.*, 1994; Swain & Pool, 2008;

Sfontouris *et al.*, 2015). Phospholipase C zeta (PLCζ), a sperm-specific factor, has been identified as the physiological agent of oocyte activation *in vivo* (Saunders *et al.*, 2002). Normally, this factor is introduced into the oocyte and cleaves membrane-bound phosphatidylinositol biphosphate to produce diacylglycerol, which initiates the zona reaction to block polyspermy, and inositol triphosphate (IP_3) (Nozawa *et al.*, 2018). Then, IP_3 binds to its receptors on the surface of the endoplasmic reticulum, which stimulates calcium release from this internal store, resulting in a persistent rise in intracellular calcium (Berridge, 2009). The resulting Ca^{2+} flux (known as an oscillatory mode), progressive decline, and eventual termination of calcium oscillations typically occur when pronuclei are formed (Marangos *et al.*, 2003). Thus, calcium is recognized as a pivotal signal for successful oocyte activation and the onset of embryogenesis (Ramadan *et al.*, 2012). *In vivo* and *in vitro*, changes in Ca^{2+} fluxes happen until the zygote prepares for the first cell division, when a spontaneous Ca^{2+} signal arises, triggering cleavage to the 2-cell stage (Swanson *et al.*, 1997; Dai *et al.*, 2014). For subsequent embryonic development, a close correlation between cell division and Ca^{2+} availability has been reported. Apart from this main Ca^{2+} trigger, there is increasing evidence that oscillating levels of proteins are also involved in mitosis (Dai *et al.*, 2014).

To overcome fertilization failure due to sperm-related problems and/or oocyte activation deficiency after ICSI, several methods such as electrical oocyte activation or modified ICSI techniques have been applied successfully to “rescue” oocyte activation (Vanden Meerschaut *et al.*, 2012). In addition to chemical artificial

activating substances for human oocytes activation, calcium ionophores such as calcimycin (A23187), ionomycin, or a combination of two ionophores are the most popular (Ebner *et al.*, 2012). A review of the literature reveals that these activators are used mainly in case reports or used to activate failed fertilized oocytes after ICSI. Therefore, the aim of this study was to evaluate the efficiency of using A23187 on the rates of fertilization, cleavage, embryo development, and pregnancy after ICSI for couples with oligoasthenoteratozoospermia (OAT) and/or with female factor aetiology such as advanced age, unexplained aetiology, polycystic ovary syndrome (PCOS), or primary ovarian insufficiency (POI) who failed to achieve pregnancy in more than two fresh cycles or with a combination of the above factors.

Materials and methods

Patients

Data were collected from a single outpatient fertility/IVF clinic's records (Reproductive Medicine Center, The First Affiliated Hospital of Anhui Medical University). In our center, couples with male and/or female factor infertility were routinely offered ICSI-AOA according to the following criteria: (1) patients with severe teratozoospermia, such as acephalicspermatozoa syndrome, globozoospermia, or small acrosome sperm; (2) patients who had failed fertilization after one ICSI procedure without oocyte abnormality; or (3) patients with embryo developmental problems [complete embryo developmental arrest, complete developmental delay (no morula/blastocyst on Day 5), and/or reduced blastocyst formation on Day 5 (≤15%) and even recurrent implantation failure] in the previous two IVF/ICSI cycles.

All couples in our cohort had failed pregnancy in more than two fresh cycles. In addition, couples presenting male factor aetiology with severe OAT or female factor aetiology with advanced age, unexplained infertility, PCOS, POI, or both with a combination of the above factors were enrolled in our study. As shown in Figure 1, 796 couples were enrolled in this retrospective analysis. Informed consent was obtained from all patients. To evaluate the effect of AOA by calcium ionophore on fertilization, embryo development, and pregnancy outcomes of couples with various reproductive problems, 374 couples with severe OAT and 307 couples with advanced maternal age (n = 93), unexplained infertility (n = 141), PCOS (n = 43), or POI (n = 30) were included. With respect to the male factor, couples were excluded from this part if there were clinically abnormal female factors (advanced maternal age, ovulatory, tubal, endometrial, and PCOS/POI). A further 115 infertile couples diagnosed with male and female factors were counselled to undergo a subsequent ICSI cycle combined with AOA using Ca^{2+} ionophore. Couples with a chromosomal abnormality in either partner were excluded. The previous cycles of the same patients were included as the control group.

Clinical outcomes refer only to the fresh embryo transfer cycles or the first frozen embryo transfer cycles within 6 months after oocyte retrieval. Cases with abnormal karyotypes or Y chromosome microdeletions were excluded from the cohort. The study was approved by the institutional ethics review board of the First Affiliated Hospital of Anhui Medical University (PJ-2015-11-16).

Oocyte retrieval, preparation, and insemination

Oocyte retrieval, preparation, and insemination were performed according to previously published guidelines (Darwish & Magdi, 2015). The media used in this study were as described in our previous report (Zhang *et al.*, 2017).

ICSI and calcium ionophore oocyte activation

In the AOA cycles, all mature oocytes from the participants were exposed to 5 μmol/L A23187 immediately after ICSI for 15 min. After a three-step washing procedure, *in vitro* culture was performed up to the blastocyst stage and then blastocysts were transferred to the uteri of recipients or cryopreserved for transfer in the next cycle.

Zygote and embryo assessment

Quality assessment of oocytes, zygotes, and embryos was performed according to the routine guidelines described in our previous study (Zhang *et al.*, 2017).

The following parameters were used to compare conventional ICSI cycles with ICSI-AOA cycles of the same patients: cleavage rate (number of cleaved zygotes/number of fertilized oocytes), blastocyst formation rate (number of blastocysts/number of cleaved zygotes), high-quality blastocyst formation rate (number of high-quality blastocysts/number of cleaved zygotes), and implantation rate (number of gestational sacs/number of embryos transferred). The previous cycles without AOA were considered a suitable control group because the cycle characteristics and laboratory variables had not changed.

Embryo transfer

Under transvaginal ultrasound guidance, one or two embryos, according to the quality of embryo and the age of the woman, were transferred into the uterus of each patient.

All patients received luteal support with 600 mg of vaginally administered micronized progesterone (Utrogestan; Besins-International Laboratories, Israel). Fourteen days after embryo transfer, serum hCG levels were measured to confirm successful embryo implantation, which was considered a positive biochemical pregnancy. Clinical pregnancy was defined as a sac visible by ultrasonography with a fetal heartbeat 7 weeks after embryo transfer.

Statistical analysis

To assess differences between the study and control group, descriptive statistics were expressed using mean ± standard deviation for normally distributed variables, and Student's *t*-test was used for measurement data using IBM SPSS Statistics for Windows (version 20.0; IBM Corp., Armonk, NY, USA). Categorical variables were analyzed by χ^2 test. $P < 0.05$ was considered statistically significant and all statistical tests were two-tailed.

Results

General characteristics of AOA and controls in each subgroup

As shown in Table 1, no significant differences were observed between AOA and controls in each subgroup with respect to male and female ages, number of retrieved oocytes, or number of metaphase II oocytes.

Infertile couples with male factor aetiology

To evaluate the effect of AOA by calcium ionophore on fertilization, embryo development, and treatment outcomes of couples with severe male factor aetiology, 374 couples meeting the inclusion criteria underwent ICSI-AOA cycles. As shown in

Figure 2, we detected a significant increase in fertilization rate following AOA for OAT patients, as well as increased rates of blastulation (Table 2) and high-quality blastocysts (Figure 3). In terms of outcome, not only was a significant improvement from the cleavage stage to blastocyst transfers observed but also significantly increased rates of implantation (Figure 4), clinical pregnancy (Figure 5), and live births in ICSI-AOA cycles compared with previous control cycles (Table 2). In addition, ionophore treatment decreased the rates of miscarriage (negative HA) and miscarriage (positive HA (HA, heart activity)) significantly (Table 2).

Infertile couples with female factor aetiology

Couples with severe male factor infertility are obvious candidates for ICSI+AOA. However, in our center, a history of two previous fresh cycles with failed IVF using fresh oocytes or absence of prior pregnancy because of female aetiologies also elicited consideration for ICSI+AOA treatment. To understand the fundamental contributions of oocyte activation with calcium ionophore after ICSI in different groups, couples were further divided according to female aetiologies, such as advanced age, unexplained infertility, and PCOS/POI. As summarized in Figure 2, no significant differences were found for fertilization rates in these three subgroups. All additional embryo development outcomes including cleavage, blastulation, and high-quality blastocyst formation rates were no different between ICSI and ICSI+AOA groups with respect to women of advanced age or with PCOS/POI except for the high-quality blastocyst formation rate of women with PCOS (Table 2 and Figure 3). However, rates of implantation (Figure 4), positive β-hCG, clinical pregnancy, and live birth

(Table 2 and Figure 5) were markedly increased compared with that in previous control cycles except for clinical pregnancy rate and live birth rate of women with POI.

Interestingly, for young patients (age <35 years) with unexplained infertility who had undergone more than two failed treatment cycles, the rates of blastulation (Table 2) and high-quality blastocyst formation (Figure 3) were significantly improved. However, there were no significant differences in fertilization (Figure 2) or cleavage rates (Table 2) between the historical control cycle and ionophore cycle. Furthermore, the rates of implantation, positive β-hCG, clinical pregnancy, and live birth were as follows: 10.5 ± 12.8 versus 50.5 ± 21.4, $P < 0.001$; 16.3 versus 64.8, $P < 0.001$; 6.1 versus 45.1, $P < 0.001$; and 6.1 versus 45.1, $P < 0.001$, for control and ionophore cycle, respectively, which were highly increased compared with control groups (Figures 4 and 5, Table 2).

Infertile couples with both male and female factor infertility

To compare with previous failed cycle attempts without AOA, 115 couples with male and female factors were enrolled in the study. The results of undergoing ICSI cycles for the same patients combined with AOA using A23187 and control cycles are presented. Compared with the historical control cycle, the rates of fertilization (Figure 2) and cleavage (Table 2) did not differ significantly with respect to treatment, whereas rates of blastulation (Table 2) and high-quality blastocysts (Figure 3) improved significantly. Furthermore, the rates of implantation (Figure 4), positive β-hCG ($P < 0.001$), clinical pregnancy (Figure 5), and live birth ($P < 0.001$) were

greatly improved by AOA (Table 2).

Discussion

Our study is the first to include a large sample size of couples undergoing ICSI and AOA among individual infertile couples with male or female factor aetiology or a combination of both. We not only evaluated oocyte fertilization but also embryo development outcomes, including cleavage, blastulation, and high-quality blastocyst formation rates between the ICSI and ICSI-AOA groups. Pregnancy outcomes, including embryo implantation, clinical pregnancy, and live birth rates, were also analyzed.

In the field of assisted reproduction technology (ART), the application of calcium (Ca^{2+})-dependent oocyte activation has been studied in depth. Over the past decades, AOA induced by a number of stimuli or combining calcium ionophore treatment with ICSI has been successfully used in cases of globozoospermia or other severe forms of teratozoospermia (Moaz *et al.*, 2006; Nasr-Esfahani *et al.*, 2008). In addition, analysis of fertilization and clinical outcomes with respect to sperm from ejaculated normal, ejaculated-oligo-astheno-terato, or extracted-testicular spermatozoa of patients with complete or virtually complete fertilization failure history, AOA increased the rates of fertilization and clinical pregnancy significantly regardless of sperm characteristics (Yoon *et al.*, 2013). In another study, the efficiency of AOA with A23187 on ICSI cycles was evaluated using spermatozoa from different sources (ejaculated sperm, epididymal sperm, and testicular sperm); AOA had no effect on embryo development and clinical outcomes for all sperm origin groups. However, for

young patients, AOA could promote embryo development when ejaculated or epididymal spermatozoa were used and increase the rate of implantation when ejaculated spermatozoa were injected (Borges *et al.*, 2009). These clinical findings indicate that AOA can improve fertilization rate, embryo quality, and clinical outcomes of patients with poor fertilization history when ejaculated sperm is used. The findings also suggest that not only spermatozoa but also the oocyte plays a role in oocyte activation. These reports were in accordance with our results that AOA improved the fertilization rate of patients with OAT not in other subgroups. This may be explained by a relationship between PLCζ and the function of oocyte activation. PLCζ, expressed in the perinuclear theca of spermatozoa, appears to play an important role in inducing intracellular calcium oscillations via an inositol-1,4,5-triphosphate (IP_3)-mediated pathway (Coward *et al.*, 2005; Jones, 2005; 2007; Grasa *et al.*, 2008) (Swann *et al.*, 2006). Immunodepletion of PLCζ from sperm extracts, the knockdown or knockout of PLCζ in mouse models, and sperm fractionation studies have suggested that PLCζ which affect the sperm's ability is able to induce Ca^{2+} oscillations in the oocyte (Saunders *et al.*, 2002; Fujimoto *et al.*, 2004; Knott *et al.*, 2005; Kurokawa *et al.*, 2005; Kurokawa *et al.*, 2007; Nozawa *et al.*, 2018). In addition, recent studies have shown that infertile patients with fertilization failure following ICSI and were unable to induce Ca^{2+} oscillations presented with defect or lacking of PLCζ expression (Yoon *et al.*, 2008; Heytens *et al.*, 2009; Taylor *et al.*, 2010; Tavalaee *et al.*, 2018; Shang *et al.*, 2019). Considering the observed properties of PLCζ in oocyte activation, therefore, abnormal form or function of PLCζ may be the

main cause of certain types of male factor infertility and oocyte activation failure.

With respect to the present study, compared with previous ICSI cycles, AOA treatment not only significantly improved the rates of blastulation and high-quality blastocysts in patients with OAT, PCOS, unexplained infertility, and both factor subgroups, but also increased the rates of implantation and positive β-hCG, even in patients with advanced age and POI/PCOS subgroups, as well as the rates of clinical pregnancy and live birth except in the POI subgroup. Additionally, the rate of miscarriage (negative HA) was decreased in the OAT, PCOS, and unexplained infertility subgroups. These clinical results may be consistent with those of a recent prospective multicenter study, which showed that treatment with Ca^{2+} ionophore improved embryo development and outcome in cases with previous developmental problems (Ebner *et al.*, 2015). Regarding these conclusions, Ca^{2+} oscillations may not involve only the short-term process in the initiation and completion of early events of oocyte activation but might also enhance embryo development in long-term (Bos-Mikich *et al.*, 1997; Ozil & Huneau, 2001; Ducibella *et al.*, 2006; Levin *et al.*, 2012). Researchers have found that a physiological or artificial lack of Ca^{2+} signal during mouse and human egg activation impairs preimplantation development, blastocyst quality, and gene expression profiling (Sousa *et al.*, 1996; Wong *et al.*, 2005; Rogers *et al.*, 2006). In addition, it has been shown that not only the implantation and post-implantation development but also the long-term fetal morphology and weight variation in offspring are influenced by altered Ca^{2+} signaling patterns (Ozil & Huneau, 2001; Ozil *et al.*, 2006). With respect to how promoting

Ca^{2+} increases during the events of oocyte activation might affect development many months later, given the important role of Ca^{2+} oscillations in oocyte activation, there is an explanation that these series of repetitive oscillations, which persist only for a few hours and terminate around pronucleus formation, make a long-term development impact, may via remodeling the chromosomes, chromatin structure and reprogramming of gene expression that occur at later stages (Thompson *et al.*, 1995; Schultz *et al.*, 1999; Wolffe & Guschin, 2000). The exact mechanism should be further examined.

Even though we have demonstrated that AOA can improve fertilization, embryo development, and blastocyst quality, we found no improvements in fertilization, blastulation, or high-quality blastocysts when a calcium ionophore was applied in patients with advanced age and POI. Although the Ca^{2+} influx ability of aged oocytes from naturally aged women is severely damaged, the capability to mount a normal sperm- and PLCζ-induced Ca^{2+} oscillatory property is virtually unaffected (Haverfield *et al.*, 2016). These findings demonstrate that factors other than oocyte activation failure affect infertility in women of advanced age. It is well established that oocyte aneuploidy may be the underlying result of oocyte aging and a major cause of age-related infertility (Hassold & Hunt, 2001; Jones & Lane, 2013), and the prolonged lifespan of oocytes might contribute to development of other cellular defects. Indeed, age-related defects in mitochondrial DNA arrangement (Barritt *et al.*, 2000), accompanied by mitochondrial dysfunction (Eichenlaub-Ritter *et al.*, 2011; Udagawa *et al.*, 2014) and alterations in gene expression profiles (Pan *et al.*, 2008; Grondahl *et*

al., 2010) have been reported, which may be the main causes of infertility in women of advanced age.

AOA has been successfully used in cases of failed fertilization after ICSI for about 20 years; the safety of oocyte activation by calcium ionophore was of utmost concern with respect to birth defects. Most ART techniques, including ICSI, prolonged *in vitro* culture, and cryopreservation are known to be related to altered gene expression (Giritharan *et al.*, 2010; Monzo *et al.*, 2012). However, the effect of AOA on gene expression seems to be less than expected. Compared with ICSI alone, gene expression profile following of ICSI-AOA treatment was similar to that of conventional IVF, revealing that AOA effectively mimics part of the events triggered by sperm entry at the genetic level (Bridges *et al.*, 2011). Other evidence shows that AOA did not result in increased risk to the physical and mental health of 79 children from the Department of Reproductive Biotechnology (Isfahan, Iran) (Deemeh *et al.*, 2015) and 5 children from Kyono ART Clinic (Japan) (Kyono *et al.*, 2008) born through AOA. A retrospective cohort study of 83 children born after AOA revealed that the birth defect type (chromosomal aberration or structural malformations), malformation type (heart, urogenital, and limb), birth weight, and gestational week at time of delivery were not significantly different (Netanella *et al.*, 2016). Moreover, a continuous follow-up of 21 children undergone AOA aged 3 to 10 years showed that neonatal and neurodevelopmental outcomes were within expected ranges, as was their language development (D'Haeseleer *et al.*, 2014; Ebner & Montag, 2016). Overall, more prospective studies with a larger number of patients are needed to confirm the

observations reported to date and to further assess the safety of AOA.

Although our findings are interesting, the present study has some limitations, especially there was no randomization of patients or oocytes that would show the effects are unequivocally due to ionophore treatment. The POI and PCOS subgroups had only a small number of patients in each group. Therefore, a prospective multicenter study with a randomized control and a larger number of patients is needed to give the definitive answers.

In summary, our data indicate that oocytes subjected to AOA using the calcium ionophore A23187 resulted in better fertilization, embryonic development, and clinical pregnancy rates in some subgroups of infertile couples. Our results raise the possibility that failure of fertilization, poor-quality blastocysts in previous cycles, or failure of pregnancy more than two fresh cycles seen among some types of infertile couples result from deficient Ca^{2+} signalling during oocyte activation.

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Authors' roles

Designed the study: MRL, DZ, and BLC. Acquisition of data and performed clinical assessments: DD, YH, RFX, DMJ, WWZ, HJZ, YJL and JYW. Analyzed the data: MRL, XJH, QL and RFX. Wrote the manuscript: MRL, YXC, ZLW and PZ. Edited

the manuscript: DZ, XJH, ZGZ. Supervised the study: ZGZ. All authors read and approved the final manuscript.

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Conflict of interest

All authors declare that they have no conflict of interest.

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Figure Legends

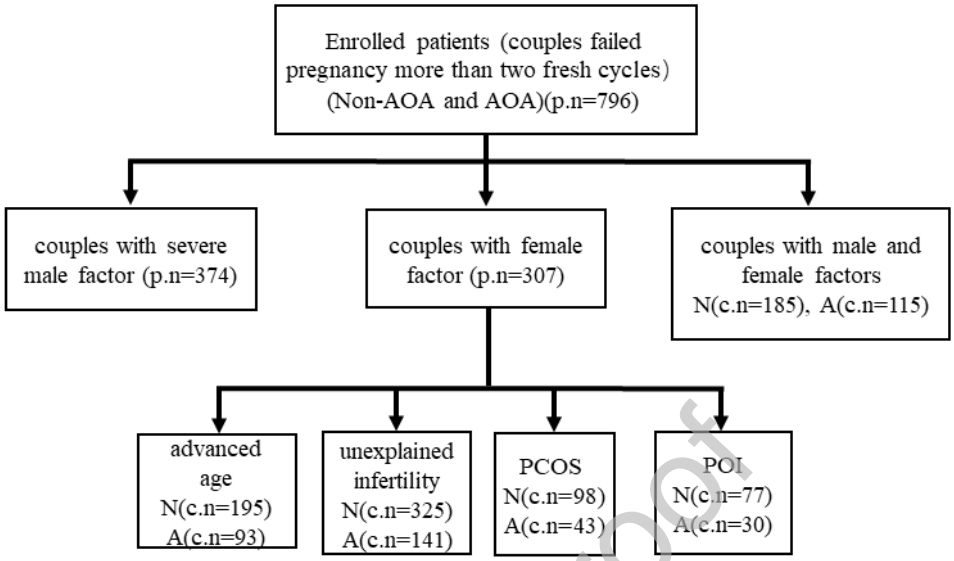

Figure 1. Experimental workflow. AOA, artificial oocyte activation; p.n, patient number; c.n, cycle number; N, non-AOA; A, AOA treatment; PCOS, polycystic ovary syndrome; POI, primary ovarian insufficiency.

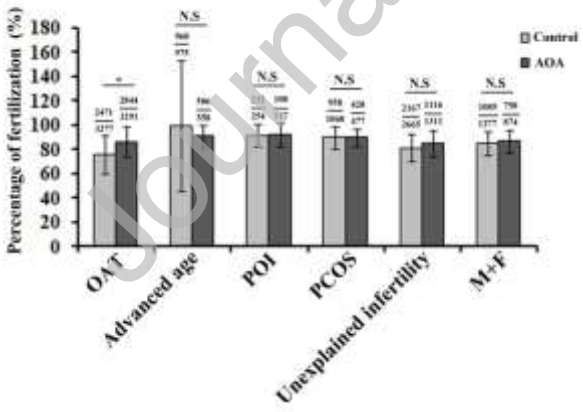

Figure 2. A comparison of fertilization rate between AOA and controls in selected subgroups of infertile couples. Compared with controls, the fertilization rate was significantly increased with AOA treatment for patients with OAT. However, there were no statistically significant differences between AOA and controls in other

subgroups. Control, historical control cycles; AOA, artificial oocyte activation treatment cycles; OAT, oligoasthenoteratozoospermia; advanced age, patients with advanced age; POI, patients with primary ovarian insufficiency; PCOS, patients with polycystic ovary syndrome; unexplained infertility, patients with unexplained infertility; M+F, both male and female factors.

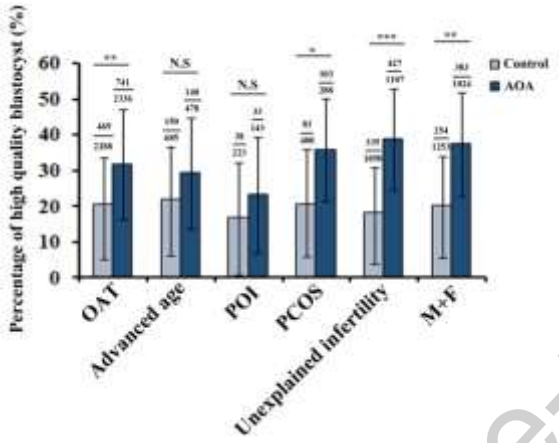

Figure 3. A comparison of high-quality blastocyst rate between AOA and controls in selected subgroups of infertile couples. Compared with controls, the high-quality blastocyst rate was increased significantly with AOA treatment for patients with OAT, PCOS, unexplained infertility, or both male and female factors. However, there were no statistically significant differences between AOA and controls in patients with advanced age or POI subgroups. Control, historical control cycles; AOA, artificial oocyte activation treatment cycles; OAT, oligoasthenoteratozoospermia; advanced age, patients with advanced age; POI, patients with primary ovarian insufficiency; PCOS, patients with polycystic ovary syndrome; unexplained infertility, patients with unexplained infertility; M+F, both male and female factors.

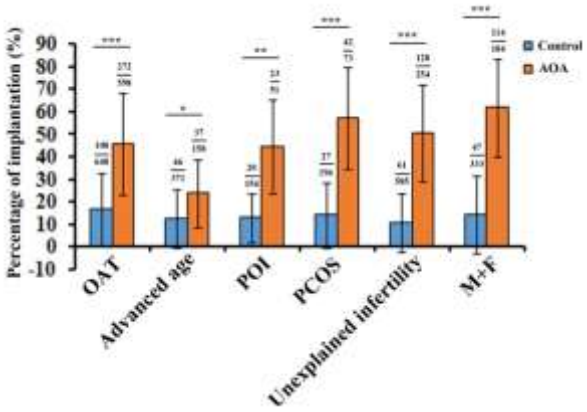

Figure 4. A comparison of implantation rate between AOA and controls in selected subgroups of infertile couples; there were statistically significant differences between AOA and controls in all subgroups. Control, historical control cycles; AOA, artificial oocyte activation treatment cycles; OAT, oligoasthenoteratozoospermia; advanced age, patients with advanced age; POI, patients with primary ovarian insufficiency; PCOS, patients with polycystic ovary syndrome; unexplained infertility, patients with unexplained infertility; M+F, both male and female factors.

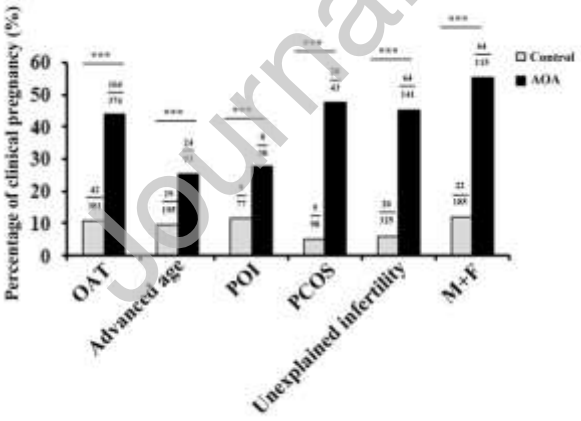

Figure 5. A comparison of clinical pregnancy rate between AOA and controls in selected subgroups of infertile couples; there was a significant increase in AOA treatment group for patients with OAT, advanced age, PCOS, unexplained infertility, or with both male and female factors except for with POI subgroup. Control, historical

control cycles; AOA, artificial oocyte activation treatment cycles; OAT, oligoasthenoteratozoospermia; advanced age, patients with advanced age; POI, patients with primary ovarian insufficiency; PCOS, patients with polycystic ovary syndrome; unexplained infertility, patients with unexplained infertility; M+F, both male and female factors.

Table 1. General characteristics of the artificial oocyte activation (AOA) and the historical control cycles for each subgroup.

Parameters \ Subgroup		OAT	Advanced age	POI	PCOS	Unexplained infertility	M+F
Cycles	Control	381	195	77	98	325	185
	AOA	374	93	30	43	141	115
Male age (yr)	Control	33.1±2.8	42.5±3.1	41.3±4.0	30.8±1.9	31.2±2.0	31.5±3.8
	AOA	34.8±3.0	45.5±2.9	42.5±3.7	32.6±1.8	32.9±2.4	32.9±4.0
Female age (yr)	Control	31.9±2.6	40.2±1.7	38.7±3.1	29.1±2.0	30.9±1.4	31.0±3.3
	AOA	33.4±2.8	43.3±2.7	39.9±3.9	30.9±1.9	32.6±1.5	32.8±3.7
No. of retrieved oocytes	Control	9.7±3.0	5.7±1.5	3.6±1.1	12.0±4.0	10.9±3.0	7.7±2.8
	AOA	10.1±3.4	6.9±2.7	4.4±2.0	13.0±4.4	11.7±3.2	8.2±4.1
No. of metaphase II	Control	8.6±2.9	5.0±2.2	3.3±1.0	10.9±4.0	8.2±2.6	6.9±2.6
	AOA	8.8±3.0	6.0±2.4	3.9±1.8	11.1±3.9	9.3±2.5	7.6±2.8

Values are presented as mean ± standard deviation or number.

Control, historical control cycles; AOA, artificial oocyte activation treatment cycles; OAT, oligoasthenoteratozoospermia; advanced age, patients with advanced age; POI, patients with primary ovarian insufficiency; PCOS, patients with polycystic ovary syndrome; unexplained infertility, patients with unexplained infertility; M+F, both male and female factors.

The data of historical control cycle was the average of two or more previous cycles of same patients in the AOA treated group.

Table 2. Effect of artificial oocyte activation using calcium ionophore in selected subgroups of infertile couples.

Parameters \ Subgroup		OAT	Advanced age	POI	PCOS	Unexplained infertility	M+F
Cleavage (%)	Control	97.0±6.8	88.2±14.9	93.7±10.2	95.3±10	97.1±7.7	93.5±11.6
	AOA	98.0±9.2	92.7±10.2	98.9±3.0	97.3±3.0	97.7±4.5	95.1±7.6
Blastulation (%)	Control	41.9±15.8	54.4±18.6	63.2±18.8	51.6±16.0	37.9±14.9	43±18
	AOA	56.2±15.0[c]	53.9±17.0	66.7±14.5	52.5±10.1	56.7±12.4	61.8±15.1[c]
No. of embryos transferred	Control	1.7±0.2	1.9±0.3	2±0.2	2.0±0.2	1.8±0.3	1.8±0.3
	AOA	1.6±0.3[a]	1.7±0.2	1.7±0.2[b]	1.7±0.3	1.8±0.2	1.6±0.2
Positive β-hCG (%)	Control	23.9	17.3	26.9	26.3	16.3	16
	AOA	53.8[c]	34.6[c]	61.1[b]	66.7[c]	64.8	72[c]
Miscarriage (negative HA) (%)	Control	36.4	11.1	28.6	40	37.5	25
	AOA	15.3[c]	5.3	9.1	7.1[a]	15.2	8.8[b]
Miscarriage (positive HA) (%)	Control	18.2	33.3	28.6	40	25	0
	AOA	7.0[a]	26.3	45.5	21.4	15.2	14.7
Multiple pregnancy rate (%)	Control	9.1	11.1	14.3	20	12.5	45
	AOA	14.0	5.3	18.2	21.4	30.4	35.3[a]
Live birth rate (%)	Control	10.9	9.6	11.5	5.3	6.1	12
	AOA	43.8[c]	25.5[c]	27.8	47.6[c]	45.1	55.3[c]

Values are presented as mean ± standard deviation or number (%).

Control, historical control cycles; AOA, artificial oocyte activation treatment cycles; OAT, oligoasthenoteratozoospermia; advanced age, patients with advanced age; POI, patients with primary ovarian insufficiency; PCOS, patients with polycystic ovary syndrome; unexplained infertility, patients with unexplained infertility; M+F, both male and female factors.

The data of historical control cycle was the average of two or more previous cycles of same patients in the AOA treated group. a: $P < 0.05$; b: $P < 0.01$; c: $P < 0.001$.

Mingrong lv received her PhD in Cell Biology from University of Science and Technology of China in 2015. Since then, she joined in the Reproductive Medicine Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Anhui Medical University. Her research has focused on molecular mechanisms of gamete abnormality.

Key Message: Application of A23187 led to increased rates of fertilization, blastulation, and high-quality blastocyst formation in certain types of infertile couples and of implantation, clinical pregnancy, and live birth in most types.