Archives of Gynecology and Obstetrics (2019) 300:1083–1092
https://doi.org/10.1007/s00404-019-05298-3

Pregnancy and neonatal outcomes of artificial oocyte activation in patients undergoing frozen–thawed embryo transfer: a 6-year population-based retrospective study

Bin Li[1] · Yiwen Zhou[2] · Zhiguang Yan[1] · Menghui Li[1] · Songguo Xue[2] · Renfei Cai[1] · Yonglun Fu[1] · Qingqing Hong[1] · Hui Long[1] · Mingru Yin[1] · Tong Du[1] · Yun Wang[1] · Yanping Kuang[1] · Zheng Yan[1] · Qifeng Lyu[1]

Received: 30 May 2019 / Accepted: 5 September 2019 / Published online: 16 September 2019

Abstract

Purpose To evaluate the impact of artificial oocyte activation (AOA) in pregnancy and neonatal outcomes in infertile patients undergoing cryopreserved embryo transfer.

Method This retrospective study included 5686 patients' transferred embryos from routine intracytoplasmic sperm injection (ICSI) and 194 patients' transferred embryos from ICSI combined with AOA (ICSI-AOA) from January 2011 to December 2016. Pregnancy and neonatal outcomes of couples undergoing routine ICSI or ICSI-AOA were analyzed before and after propensity score matching. Artificial oocyte activation was performed with ionomycin.

Results The pregnancy outcomes showed no significant difference in the rates of biochemical pregnancy, clinical pregnancy, implantation, miscarriage, ectopic pregnancy, multiple pregnancy, and live births between the routine ICSI and ICSI-AOA groups before and after propensity score matching, respectively. The assessment of neonatal outcomes showed no statistically significant differences in the birth defect rate, birth weight, gestational age, preterm birth rate, early-neonatal death rate, and fetal sex ratio between the two groups, and similar results were also observed in the two matched cohorts.

Conclusion Artificial oocyte activation with ionomycin does not adversely affect pregnancy and neonatal outcomes in patients undergoing frozen–thawed embryo transfer, which is beneficial to clinicians counseling patients on the risks of artificial oocyte activation.

Keywords Artificial oocyte activation · Frozen–thawed embryo transfer · Birth defect

Introduction

Oocyte activation is an important step in normal fertilization and subsequent embryogenesis. This process is induced by calcium oscillations which increase the intracellular calcium levels in the oocyte, essential for a series of changes to the nucleus and cytoplasm before pronuclear formation [1]. Artificial oocyte activation (AOA) has been used to effectively increase the clinical outcomes in infertile couples with null or low fertilization [2–6], poor embryo development [7], and severe male factor infertility [8–12].

At present, the application of a chemical reagent is the main choice for AOA treatment. Two calcium ionophores, ionomycin [11] and A23187 [4–7], are the most widely used AOA chemical reagents. Although the exposure time of calcium ionophore is short and its concentration in the culture medium is low, the potential adverse effects of AOA on post-implantation embryo and fetal development

Bin Li, Yiwen Zhou and Zhiguang Yan contributed equally to this work.

✉ Zheng Yan
yanzheng369@163.com

✉ Qifeng Lyu
lyuqifeng@126.com

[1] Department of Assisted Reproduction, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200011, China

[2] Department of Assisted Reproduction, Shanghai East Hospital, Shanghai Tongji University School of Medicine, Shanghai 200120, China

should be further studied. Montag et al. reported that 25 babies were born after AOA with calcium ionophore A23187 without congenital malformation [4]. In two prospective multicenter studies, 32 and 35 healthy children of AOA, with calcium ionophore A23187, were born from couples with fertilization problems and severe male factor infertility [6, 13]. So far, pregnancy and neonatal outcomes, especially birth defects of AOA application, are scarcely reported [3–8, 11, 13]. Additionally, all infants in published AOA studies were born from fresh embryo transfer (ET), and none of the babies born from AOA in frozen–thawed embryo transfer (FET) were reported.

Here, we designed this study with the objective of evaluating the effect of AOA on pregnancy and neonatal outcomes of infertile patients undergoing FET: (1) pregnancy outcomes were compared between patients with routine intracytoplasmic sperm injection (ICSI) and ICSI-AOA; (2) neonatal outcomes were compared between patients with routine ICSI and ICSI-AOA. These results would be beneficial to clinicians counseling patients on the risks of AOA.

Material and methods

Study setting and patients

This retrospective cohort study was conducted by the Department of Assisted Reproduction of the Ninth People's Hospital of Shanghai, Jiaotong University School of Medicine. Pregnancy and neonatal outcomes of patients undergoing routine ICSI and ICSI-AOA, were evaluated from January 2011 to December 2016. The following patients were excluded: (1) women who transferred mixed embryos from ICSI-AOA cycles and previous routine ICSI cycles and (2) women who were lost to follow-up. Based on the published literature, AOA was performed using the following criteria: (1) ICSI fertilization rate ≤ 50 (in- and out-hospital cases) [4–6]; (2) good quality embryo rate ≤ 30% (in- and out-hospital cases); (3) the presence of severe oligoasthenoteratozoospermia (OAT) [8, 11]; (4) surgically retrieved sperms from testicular sperm aspiration (TESA) or percutaneous epididymal sperm aspiration (PESA) [9, 10, 12, 13]. Ionomycin was used for AOA.

This study was approved by the Ethics Committee (institutional review board) of Shanghai Ninth People's Hospital affiliated to Shanghai Jiao Tong University School of Medicine. All relevant international ethical codes were followed, including the Helsinki Declaration. All participants provided informed consent after receiving counseling concerning infertility treatments.

Analysis

A total of 5880 patients undergoing FET were analyzed: (1) the routine ICSI group included 5686 patients with transferred embryos from routine ICSI; (2) ICSI-AOA group included 194 patients with transferred embryos from ICSI combined with AOA (Fig. 1). Data were collected from the first FET cycle of each patient and repeated cycles from the same patient were not used.

This study comprised two parts (Fig. 1): in the first, we compared pregnancy outcomes of FET in the routine ICSI and ICSI-AOA groups; in the second, we compared neonatal outcomes of FET in the routine ICSI and ICSI-AOA groups. Moreover, singleton and twin births in the two cohorts were compared separately. Propensity scores were calculated using logistic regression based on patient and FET characteristics, including female age, male age, female body mass index (BMI), the duration of infertility, number of transferred embryos, number of transferred embryos per cycle and type of embryo transferred cycles (cleavage embryo and blastocyst), endometrial thickness on embryo transfer day, the type of endometrial preparation (natural cycles, stimulated cycles, and hormone therapy cycles), and causes of infertility (tubal factor infertility, polycystic ovarian syndrome, endometriosis, male factor infertility, and other). Matching without replacement was performed using propensity scores with the nearest neighbor, random matching algorithm [14]. The matched ratio of the routine ICSI group to ICSI-AOA group was 3:1.

Treatment

The procedures of ovarian stimulation, ICSI, embryo assessment, freezing, and thawing, endometrial preparation, and FET have been described in previous studies [15, 16].

Briefly, oocyte retrieval was carried out 32–36 h after maturation induction or ovarian stimulation. Retrieved oocytes were washed and cultured in human tubal fluid (HTF; Irvine Scientific, Santa Ana, CA, USA) with 10% serum substitute supplements (Irvine Scientific) after retrieval. The cumulus cells were carefully removed after 2–3 h of oocyte retrieval and oocytes were injected by ICSI within 2 h of oocyte denudation under 200× magnification on a microscope (Nikon, Japan) equipped with a warming plate (37 °C) and a manipulator (Narishige, Tokyo, Japan). Each oocyte was positioned with its polar body at the 12 o'clock position, and subsequently, an injecting pipette containing immobilized sperm was introduced at the 3 o'clock position.

Fertilization was assessed 18 h after ICSI, and normal fertilization was characterized by two distinct pronuclei.

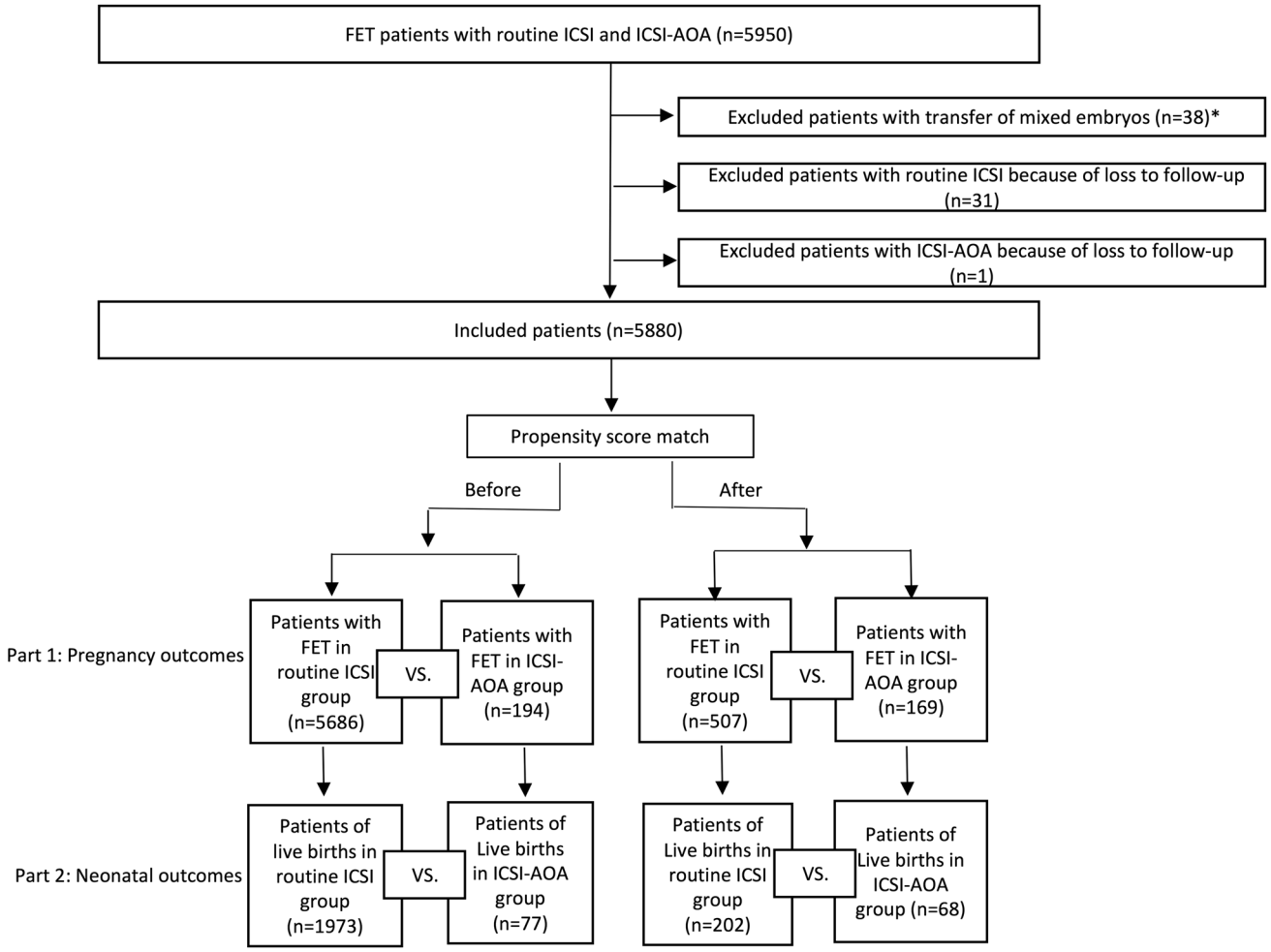

Fig. 1 Flow chart of the study. *FET* frozen–thawed embryo transfer, *ICSI* intracytoplasmic sperm injection, *AOA* artificial oocyte activation, *vs.* versus; *Patients in whom mixed embryos were transferred from ICSI-AOA and previous routine ICSI cycles were excluded

The embryos were evaluated on the third day after fertilization based on ASEBIR embryo assessment criteria [17]. Grades I and II embryos, regarded as top quality, were cryopreserved by vitrification. The remaining embryos were cultured until the blastocyst stage. During this stage, the Gardner and Schoolcraft system [18] was used to grade blastocysts. Only blastocysts with > grade 3CC were cryopreserved on day 5–7.

The cryopreserved and thawing procedures for cleavage-stage embryos or blastocysts were performed with Kitazato Vitrification Kit following the manufacturer's introduction (Kitazato, Japan) [19]. Briefly, embryos were first incubated for 10–15 min in about 300 µl equilibration solution [7.5% ethylene glycol and 7.5% dimethylsulfoxide (DMSO) and 20% synthetic serum substitute] at room temperature, and then were transferred into vitrification solution (15% ethylene glycol and 15% DMSO and 0.5 M trehalose and 20% synthetic serum substitute) to incubate for approximately 60 s, and finally, the embryos were loaded on the Cryotop strip (Kitazato, Japan) in a minimum volume, and were directly immersed into liquid nitrogen. For the thawing procedure, the strip of the Cryotop was immersed into about 500 µl 37 °C thawing solution (1.0 M trehalose and 20% synthetic serum substitute) for approximately 60 s, and then embryos were transferred in the diluent solution for 3 min and washed twice in the wash solution for 5 min, respectively, at room temperature. Finally, the embryos were incubated in culture medium supplemented with 10% serum substitute supplements (Irvine Scientific) at 37 °C, CO_2 6%.

Endometrial preparation for FET was achieved in natural cycles, stimulation cycles or hormone therapy cycles, as previously described [15]. In short, in patients with regular menstrual cycles, FET was performed in a natural cycle. For patients with irregular menstrual cycles, letrozole and, if necessary, human menopausal gonadotropin were used to stimulate monofollicular growth. In patients with a history of thin endometrium during either natural cycles or stimulation cycles, hormonal therapy was applied.

Artificial oocyte activation

AOA was performed according to a methodology previously reported by Nasr-Esfahani [20]. The oocytes were artificially activated, by exposure to 10 μM ionomycin (Sigma, St. Louis, MO, USA) for 10 min at 37 °C in 6% CO_2 and 5% O_2, after 1 h of ICSI manipulation. Following oocyte activation, the oocytes were thoroughly washed in HTF and cultured in a continuous single-culture medium (Irvine Scientific) throughout the developmental stage.

Outcome assessment

The outcome terms were defined based on the International Committee for Monitoring Assisted Reproductive Technology (ART) and the World Health Organization revised glossary of ART terminology 2009 [21], including biochemical pregnancy, clinical pregnancy, ectopic pregnancy, multiple birth, implantation rate, miscarriage rate, live birth rate, etc. Gestational age was defined as age of a fetus calculated by adding 2 weeks to the number of completed weeks. Preterm birth was defined as birth before 37 weeks of gestation. Low birth weight was defined as birth weight < 2500 g. Early-neonatal death was defined as death of a live born baby within 7 days.

Details of the assessment for birth defects have been described in previously published papers [22, 23]. Briefly, babies born in our hospitals received a routine physical examination at birth and were registered in the electronic follow-up recording system. For neonates born in other hospitals, written health reports were provided by the primary paediatrician, including information about birth dates and locations, gestational weeks, mode of delivery, birth weight, infant sex, neonatal disease, and live birth defects. For infants with birth defects, an independent pediatrician examined the case reports based on clinical experience to ensure that these infants met the inclusion criteria of the Chinese Birth Defects Monitoring Program. Live birth defects were classified according to the International Classification of Diseases 10th Revision as conditions registered in the International Statistical Classification of Diseases and Related Health Problems [24]. Minor birth defects were excluded, except those that required treatment or were disfiguring.

Statistical analysis

Quantitative data are presented as mean ± standard deviation (SD). Mean values were compared by Student's *t* test and proportions were compared by the Chi-squared test or Fisher's exact test. Values of $P < 0.05$ were considered to be significantly different. All analyses were performed using SPSS, version 23.0 software (SPSS Inc., Chicago, IL, USA).

Results

Comparison of pregnancy outcomes between the routine ICSI and ICSI-AOA groups

After exclusions, a total of 5880 patients undergoing FET were analyzed: (1) 10,608 frozen embryos were transferred to 5686 patients undergoing routine ICSI; and (2) 348 frozen embryos were transferred to 194 patients undergoing ICSI-AOA. Results showed that no significant differences were found in the rates of biochemical pregnancy, clinical pregnancy, implantation, miscarriage, ectopic pregnancy, multiple pregnancy, and live births between the routine ICSI and ICSI-AOA groups ($P > 0.05$, Table 1). However, we found that the average maternal and paternal ages were significantly lower in the ICSI-AOA group than those in the routine ICSI group (32.84 ± 5.13 vs 35.97 ± 5.47, $P < 0.001$ and 35.32 ± 6.05 vs. 38.29 ± 6.73, $P < 0.001$, respectively), and there were also significant differences in BMI, the number of embryos per transfer, type of embryo transferred cycles, type of endometrial preparation, and causes of infertility between the routine ICSI group and ICSI-AOA group ($P < 0.05$) (Table 1).

Considering the effects of differences in patients' characteristics on pregnancy outcomes, we used propensity score matching to control for the different characteristics of the two groups. We matched 507 patients from the routine ICSI group with 169 patients from the ICSI-AOA group in a 3:1 ratio (Fig. 1). The distributions of propensity scores after matching indicated a balance between the two groups, when compared to those before matching (Fig. 2). After matching, characteristics of the matched routine ICSI and ICSI-AOA groups were similar ($P > 0.05$), and no significant differences were observed in all pregnancy outcomes between the two matched groups ($P > 0.05$), which was consistent with the previous results before matching. The characteristics of the patients undergoing FET are summarized in Table 1.

Comparison of neonatal outcomes between the routine ICSI and ICSI-AOA groups

To evaluate the safety of AOA in terms of neonatal outcomes and birth defects in fetuses from patients undergoing FET, a total of 2537 babies were analyzed, including 2442 babies of the routine ICSI group and 95 babies of the ICSI-AOA group. The results showed that no significant differences were found in birth weight, gestational age, fetal sex and preterm birth rates between the two groups ($P > 0.05$, Table 2). No case of early-neonatal mortality was observed in the ICSI-AOA group, whereas six early-neonatal deaths occurred in the routine ICSI group. Furthermore, singleton and twin deliveries were analyzed separately. 1504

Table 1 Pregnancy outcomes of FET between the routine ICSI and ICSI-AOA groups

Group	Before propensity score matching		*P* value	After propensity score matching		*P*-value
	Routine ICSI group	ICSI-AOA group		Routine ICSI group	ICSI-AOA group	
No. of patients	5686	194		507	169	
Female age, years	35.97 ± 5.47	32.84 ± 5.13	**< 0.001**	33.56 ± 4.89	33.46 ± 4.67	0.822
Male age, years	38.29 ± 6.73	35.32 ± 6.05	**< 0.001**	35.79 ± 5.96	35.73 ± 5.64	0.898
BMI, kg/m^2	21.83 ± 3.10	21.29 ± 2.72	**0.008**	21.29 ± 2.46	21.23 ± 2.45	0.783
Duration of infertility, years	4.54 ± 3.33	4.53 ± 3.43	0.984	4.29 ± 3.24	4.46 ± 3.30	0.544
No. of embryos transferred	10 608	348		920	308	
No. of embryos per transfer	1.87 ± 0.34	1.79 ± 0.41	**0.016**	1.81 ± 0.39	1.82 ± 0.38	0.819
Type of embryo transferred cycles			**0.004**			0.824
Cleavage embryo transferred cycles, *n* (%)	5331 (93.76)	172 (88.66)		456 (89.94)	153 (90.53)	
Blastocyst transferred cycles, *n* (%)	355 (6.24)	22 (11.34)		51 (10.06)	16 (9.47)	
Endometrial thickness, mm	10.93 ± 2.42	10.96 ± 2.59	0.836	10.86 ± 2.44	10.88 ± 2.46	0.904
Endometrium preparation			**0.003**			0.551
Natural cycles, *n* (%)	2907 (51.13)	85 (43.81)		230 (45.36)	78 (46.15)	
Stimulated cycles, *n* (%)	1006 (17.69)	53 (27.32)		116 (22.88)	44 (26.04)	
Hormone therapy cycles, *n* (%)	1773 (31.18)	56 (28.87)		161 (31.76)	47 (27.81)	
Causes of infertility						
Tubal factor infertility, *n* (%)	2979 (52.39)	73 (37.63)	**< 0.001**	214 (42.21)	68 (40.24)	0.652
Polycystic ovary syndrome, *n* (%)	488 (8.60)	16 (8.25)	0.973	47 (9.27)	13 (7.70)	0.532
Endometriosis, *n* (%)	423 (7.40)	13 (6.70)	0.788	34 (6.71)	13 (7.70)	0.662
Male factor infertility, *n* (%)	3355 (59.00)	142 (73.20)	**< 0.001**	356 (70.22)	120 (71.01)	0.846
Other, *n* (%)	703 (12.36)	32 (16.49)	0.058	75 (14.79)	27 (15.98)	0.710
Biochemical pregnancy, %	4.54 (258/5686)	2.06% (4/194)	0.10	4.93 (25/507)	2.37 (4/169)	0.154
Clinical pregnancy, %	43.00 (2445/5686)	46.91 (91/194)	0.28	50.49 (256/507)	47.93 (81/169)	0.564
Implantation, %	29.14 (3091/10,608)	31.61 (110/348)	0.319	36.20 (333/920)	33.44 (103/308)	0.382
Miscarriage, %	17.55 (429/2445)	14.29 (13/91)	0.421	19.53 (50/256)	13.58 (11/81)	0.225
Ectopic pregnancy, %	1.72 (42/2445)	2.20 (2/91)	0.949	1.56 (4/256)	2.47 (2/81)	0.956
Multiple births, %	23.77 (469/1973)	23.38 (18/77)	0.936	31.68 (64/202)	26.47 (18/68)	0.419
Live births, %	34.70 (1973/5686)	39.69 (77/194)	0.151	39.84 (202/507)	40.24 (68/169)	0.928

Differences in mean values were compared between the groups using Student's *t* test. Differences in rates were compared between the groups using the chi-square test or Fisher's exact, as appropriate. $P < 0.05$ was considered statistically significant

No. number, *FET* frozen–thawed embryo transfer, *ICSI* intracytoplasmic sperm injection, *AOA* artificial oocyte activation, *BMI* body mass index

singletons were born in the ICSI group, and 59 were born in the ICSI-AOA group. No significant differences were found in birth weight, gestational week and fetal sex between the two groups, and similar results were observed in twin deliveries (Table 2). In terms of birth defects, twin boys with congenital anomaly (patent ductus arteriosus) were found in the ICSI-AOA group, and 31 babies with congenital defects were found in the routine ICSI group. The birth defect rate was not significantly different between the two groups (2.11% vs. 1.27%, $P > 0.05$). Types of birth defects among live-born babies are presented in Table 3.

However, we also found that there were significant differences in the average ages of both spouses and causes of infertility between the routine ICSI group and ICSI-AOA group ($P < 0.05$, Table 2). We further analyzed the neonatal outcomes in the two matched groups to control for the differences in patients' characteristics. 86 babies of the ICSI-AOA group were compared to 266 babies of the routine ICSI group, no significant differences were found in neonatal outcomes, and similar results were observed in singletons or twin deliveries, respectively ($P > 0.05$, Table 2).

Discussion

Comparisons of results between our study and previous studies

FET performed with the freeze-all policy has become increasingly popular in the recent years. The potential advantage of this strategy is that it can enhance embryo–endometrium synchrony and provide a more

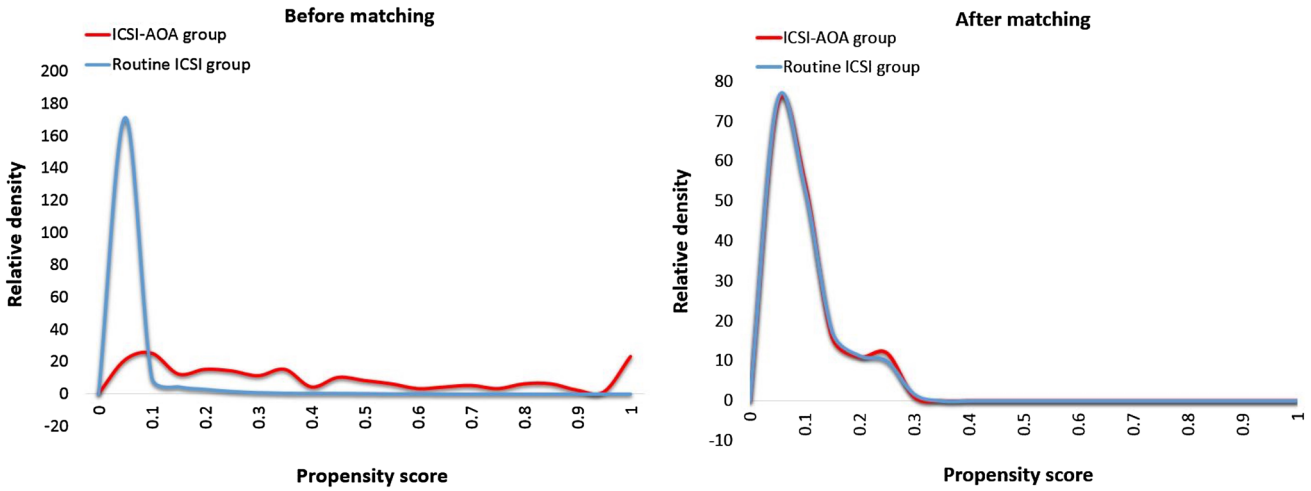

Fig. 2 Propensity score matching for patients undergoing FET in the routine ICSI and ICSI-AOA groups. *ICSI* intracytoplasmic sperm injection, *AOA* artificial oocyte activation

physiologic environment for the embryos [25, 26]. Several studies have suggested that FET is associated with better clinical outcomes than fresh ET [27, 28]. Because of these strengths, the freeze-all policy has been performed in our center. So far, the safety of AOA in infertile patients undergoing FET remains unknown. Herein, this study is designed to assess the impact of AOA on pregnancy and neonatal outcomes of infertile patients following FET.

Previous studies mainly focused on comparisons of clinical outcomes between previous ICSI cycles and subsequent ICSI-AOA cycles in the same patients [3, 4, 6]. However, it is still unclear to what extent AOA can improve pregnancy outcomes compared to routine ICSI. Here, we found that there were no significant differences in pregnancy outcomes between the routine ICSI group and ICSI-AOA group, and similar results were also observed after propensity score matching (Table 1), which suggested that AOA might yield comparable pregnancy outcomes to those of patients with routine ICSI.

At present, several studies have reported that the application of AOA may improve the clinical outcomes in patients for different infertile indications, including null or low fertilization [4–6], poor embryo development [7], and severe male factor infertility [8–12]. 38, 32, 25, 22, and 16 healthy children born following AOA were reported, showing no major congenital malformations [4, 5, 7, 8, 13]. However, the number of babies born from infertile patients undergoing AOA was still limited, more neonates should be collected to assess the safety of AOA. Herein, we summed 95 AOA babies from different indications to evaluate neonatal outcomes. Our results showed that there were no significant differences in birth weight, gestational age, fetal sex, and preterm birth between patients undergoing routine ICSI and those of ICSI-AOA. Additionally, no neonatal mortality case was found and only twin boys with patent ductus arteriosus were delivered in ICSI-AOA group. Miller et al. [29] reported that the birth defect rates were about 6% in routine ICSI group and 9% in ICSI-AOA group, which were higher than ours. Our results showed that the birth defect rates in routine ICSI group and ICSI-AOA group were 1.27% and 2.11%, respectively, which is similar to those of published papers [22, 30–32]. The reason for the difference may be that we have no information of pregnancy termination in early developmental stage and chromosomal anomalies examined with prenatal diagnosis, which led to underestimating the prevalence of birth defects [33, 34].

In the present study, we found that the average maternal ages of patients delivering babies were different between patients with ICSI-AOA and those with routine ICSI [31.57 ± 4.00 versus (vs.) 33.95 ± 4.33, $P < 0.001$, Table 2], which was similar to that in a previous study [29]. Although higher maternal age is associated with an increased risk of birth defects [35], a higher rate of birth defects was not found in the routine ICSI group. Moreover, no significant differences were also found in the two marched groups with balanced characteristics (Table 2). Thus, we speculated that the occurrence of live birth defect may not be influenced by the age factor in this study.

Currently, the most widely used chemical reagents for AOA are, Ca^{2+} ionophores, A23187 and ionomycin [36]. Nikiforaki et al. [37] assessed the effect of these two AOA compounds in human and mouse oocytes and found that ionomycin was more potent than A23187 in increasing Ca^{2+} release and oocyte activation rate. However, they did not evaluate the safety of the two AOA reagents in terms of offspring. In this study, we found that ionomycin may not increase the risk of birth defects, which is consistent with the results of, Ca^{2+} ionophore, A23187 in past studies

Table 2 Neonatal outcomes of FET between the routine ICSI and ICSI-AOA groups

Group	Before propensity score matching		*P*-value	After propensity score matching		*P*-value
	Routine ICSI group	ICSI-AOA group		Routine ICSI group	ICSI-AOA group	
No. of patients	1973	77		202	68	
Female age, years	33.95 ± 4.33	31.57 ± 4.00	**< 0.001**	31.98 ± 4.18	32.15 ± 3.67	0.769
Male age, years	36.24 ± 5.63	34.08 ± 5.43	**0.001**	34.43 ± 5.13	34.43 ± 5.18	0.995
BMI, kg/m^2	21.70 ± 3.18	21.37 ± 3.04	0.380	21.15 ± 2.23	21.33 ± 2.59	0.575
No. of embryos transferred	3766	146		377	131	
No. of embryos per transfer	1.91 ± 0.29	1.90 ± 0.31	0.706	1.87 ± 0.34	1.93 ± 0.26	0.134
Type of embryo transferred cycles			0.273			0.629
Cleavage embryo transferred cycles, *n* (%)	1833 (92.90)	69 (89.61)		180 (89.12)	62 (91.18)	
Blastocyst transferred cycles, *n* (%)	140 (7.10)	8 (10.39)		22 (10.89)	6 (8.82)	
Endometrial thickness, mm	11.14 ± 2.30	11.07 ± 2.59	0.830	10.97 ± 2.36	10.98 ± 2.52	0.991
Endometrium preparation			0.287			0.292
Natural cycles, *n* (%)	1050 (53.22)	40 (51.95)		92 (45.54)	37 (54.41)	
Stimulated cycles, *n* (%)	405 (20.53)	21 (27.27)		53 (26.24)	18 (26.47)	
Hormone therapy cycles, *n* (%)	518 (26.25)	16 (20.78)		57 (28.22)	13 (19.12)	
Causes of infertility						
Tubal factor infertility, *n* (%)	961 (48.71)	26 (33.77)	**0.01**	81 (39.90)	26 (38.24)	0.786
Polycystic ovary syndrome, *n* (%)	204 (10.34)	6 (7.79)	0.470	18 (8.87)	5 (7.35)	0.691
Endometriosis, *n* (%)	141 (7.15)	5 (6.49)	0.827	11 (5.42)	5 (7.35)	0.780
Male factor infertility, *n* (%)	1264 (64.06)	60 (77.92)	**0.013**	149 (73.40)	51 (75.00)	0.840
Other, *n* (%)	190 (9.63)	10 (12.99)	0.330	29 (14.78)	8 (11.76)	0.591
No. of children	2442	95	0.913	266	86	0.421
Single	1504 (76.23)	59 (76.62)		138 (68.32)	50 (73.53)	
Twins	938 (23.77)	36 (23.38)		128 (31.68)	36 (26.47)	
Birth weight (g)	3020.2 ± 611.51	3023.6 ± 603.50	0.958	2932.10 ± 649.20	3021.50 ± 571.40	0.255
Single	3320.0 ± 497.72	3256.7 ± 563.99	0.344	3378.21 ± 449.92	3290.40 ± 486.55	0.251
Twins	2546.2 ± 448.10	2647.9 ± 463.30	0.182	2474.73 ± 473.90	2647.92 ± 463.30	0.053
Gestational age (weeks)	37.98 ± 2.00	37.93 ± 2.40	0.847	37.87 ± 2.08	37.98 ± 2.01	0.693
Single	38.59 ± 1.60	38.33 ± 2.27	0.235	38.73 ± 1.37	38.46 ± 1.55	0.248
Twins	36.04 ± 1.94	36.67 ± 2.54	0.182	36.02 ± 2.13	36.67 ± 2.54	0.277
Sex (male/female)	1232/1210	45/50	0.556	138/128	43/43	0.762
Single	765/739	27/32	0.442	80/58	25/25	0.331
Twins	467/471	18/18	0.992	58/70	18/18	0.618
Preterm birth rate, %	16.52% (326/1973)	10.39% (8/77)	0.153	19.31% (39/202)	10.29% (7/68)	0.087
Early-neonatal death rate (%)	0.25% (6/2442)	0	1	0	0	/
Live birth defects (%)	1.27% (31/2442)	2.11% (2/95)	0.807	1.13% (3/266)	2.33% (2/86)	0.770

Differences in mean values were compared between the groups using Student's *t* test. Differences in rates were compared between the groups using the Chi-square test or Fisher's exact, as appropriate. $P < 0.05$ was considered statistically significant

No. number, *FET* frozen–thawed embryo transfer, *ICSI* intracytoplasmic sperm injection, *AOA* artificial oocyte activation, *BMI* body mass index

[4–7]. Potential risks of AOA such as mutagenic and epigenetic effects on oocytes and embryos should be investigated further. In addition, Evgenia et al. [38] reported a successful pregnancy after the calcium ionophore correction of pronuclei position in oocytes after ICSI, providing one more direction of AOA study, such as for correction of a pronuclei position.

Strengths and limitations

In previous studies, all AOA treatments were followed by fresh ET, and the sample sizes were limited [2–13]. With the increasing use of freeze-all policy, it is imperative and important to evaluate the safety of AOA in those patients undergoing FET. The main strength of this study is the first

Table 3 Types of congenital malformations among live-born infants

Type of malformation	ICD-10 code and diagnosis	Routine ICSI group, *n*	ICSI-AOA group, *n*
Circulatory system	Q21.1: atrial septal defect (7)	7	0
	Q21.3: tetralogy of Fallot (1)	1	0
	Q22.4: tricuspid atresia (0)	0	0
	Q21.103: patent foramen ovale (1)	1	0
	Q25.001: patent ductus arteriosus (3)	1	2
	Q20.3: ventricular septal defect (3)	3	0
	Q28.3: hemangioma (5)	5	0
Digestive system	0	0	0
Urinary system	N43.301: hydrocele (1)	1	0
Nervous system	0	0	0
Other malformations	Q27.3: peripheral arteriovenous malformation (2)	2	0
	Q55.002: favism (2)	2	0
	Q90.902: Down syndrome (1)	1	0
	Q38.101: ankyloglossia (2)	2	0
	P05.901: intrauterine growth retardation (0)	0	0
	Q69.901 polydactyly (2)	2	0
	Q79.8: Poland syndrome (1)	1	0
	Q36.1: cleft lip (1)	1	0
	M08.251: coxitis (1)	1	0
Total birth defects	1.30 (33/2537)	31	2

ICD-10 International classification of diseases, tenth revision, *ICSI* intracytoplasmic sperm injection, *AOA* artificial oocyte activation

investigation of pregnancy and neonatal outcomes including live birth defects in AOA patients undergoing FET. Our results showed similar pregnancy and neonatal outcomes between ICSI-AOA and routine ICSI groups. A total of 95 babies were born, which is a relatively larger number of live infants born from AOA compared to those in published papers [3–8, 11, 13]. Importantly, our neonatal outcomes indicated that the risk of live birth defects was not increased by AOA in patients undergoing FET. These findings suggest that AOA may be considered a safe method for clinical application in assisted reproductive technology.

In this study, propensity score matching was used to control for potential confounders between patients with routine ICSI and those with ICSI-AOA. It enabled us to assess the clinical outcomes independently from the patients' characteristics, which varied considerably between the two groups. This approach is valuable in studies where treatment assignment is not randomized, and it can be considered as an effective method for finding replicated allocation in conventional randomized controlled trials [14, 39]. In our case, the optimal matching proportion was 3:1 for the routine ICSI and ICSI-AOA groups (Fig. 1). As expected, the characteristics of the two groups were well matched, and the differences were effectively controlled. Similar pregnancy and neonatal outcomes were observed before and after the matching process (Tables 1, 2). The results indicated that the variances of different characteristics between the two groups had little effect on the clinical outcomes in our study.

The present study also has some limitations. First, we did not analyze the data separately according to different AOA indications due to the limited sample size. Second, information of pregnancy termination during the early developmental period and chromosomal anomalies detected with prenatal diagnosis were unavailable, which may have resulted in underestimation of the birth defect rates. Third, because of the freeze-all policy established in our center, we could not compare the pregnancy and neonatal outcomes of ICSI-AOA between ET and FET cycles.

In conclusion, the results of the present study demonstrated that AOA with ionomycin does not bring adverse effect on pregnancy and neonatal outcomes in infertile patients undergoing FET treatment. These findings are beneficial to clinicians counseling patients on the risks of AOA. Further ideal studies would be a randomized controlled trial in a multicenter setting and evaluate clinical safety regarding long-term follow-up of large numbers of children born from AOA.

Acknowledgements The authors thank Meiping Sheng and Suqun Zhang for following up with the patients and auditing the data.

Author contribution ZY and QL were involved in the conception and design, and revision of the article. BL, YZ, and ZY analyzed the data and completed the manuscript writing. SX, YW, RC, YF, QH, ML, and YK completed the collection of clinical data. TD, HL, and MY conducted the statistical analysis.

Funding This work was supported by The National Nature Science Foundation of China (Grant Numbers 81771649 and 81571486).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Kashir J, Heindryckx B, Jones C, de Sutter P, Parrington J, Coward K (2010) Oocyte activation, phospholipase C zeta and human infertility. Hum Reprod Update 16:690–703
2. Baltaci V, Ayvaz OU, Unsal E, Aktaş Y, Baltaci A, Turhan F et al (2010) The effectiveness of intracytoplasmic sperm injection combined with piezoelectric stimulation in infertile couples with total fertilization failure. Fertil Steril 94:900–904
3. Kyono K, Takisawa T, Nakajo Y, Doshida M, Toya M (2012) Birth and follow-up of babies born following ICSI with oocyte activation using strontium chloride or calcium ionophore A23187. J Mamm Ova Res 29:35–40
4. Montag M, Köster M, van der Ven K, Bohlen U, van der Ven H (2012) The benefit of artificial oocyte activation is dependent on the fertilization rate in a previous treatment cycle. Reprod Biomed Online 24:521–526
5. Yoon HJ, Bae IH, Kim HJ, Jang JM, Hur YS, Kim HK et al (2013) Analysis of clinical outcomes with respect to spermatozoan origin after artificial oocyte activation with a calcium ionophore. J Assist Reprod Genet 30:1569–1575
6. Ebner T, Montag M, Montag M, Van der Ven K, Van der Ven H et al (2015) Live birth after artificial oocyte activation using a ready-to-use ionophore: a prospective multicentre study. Reprod Biomed Online 30:359–365
7. Ebner T, Oppelt P, Wöber M, Staples P, Mayer RB, Sonnleitner U et al (2015) Treatment with Ca^{2+} ionophore improves embryo development and outcome in cases with previous developmental problems: a prospective multicenter study. Hum Reprod 30:97–102
8. Mansour R, Fahmy I, Tawab NA, Kamal A, El-Demery Y, Aboulghar M et al (2009) Electrical activation of oocytes after intracytoplasmic sperm injection: a controlled randomized study. Fertil Steril 91:133–139
9. Borges E, de Almeida Ferreira Braga DP, de Sousa Bonetti TC, Iaconelli A, Franco JG (2009) Artificial oocyte activation with calcium ionophore A23187 in intracytoplasmic sperm injection cycles using surgically retrieved spermatozoa. Fertil Steril 92:131–136
10. Kang HJ, Lee SH, Park YS, Lim CK, Ko DS, Yang KM et al (2015) Artificial oocyte activation in intracytoplasmic sperm injection cycles using testicular sperm in human in vitro fertilization. Clin Exp Reprod Med 42:45–50
11. Deemeh MR, Tavalaee M, Nasr-Esfahani MH (2015) Health of children born through artificial oocyte activation: a pilot study. Reprod Sci 22:322–328
12. Borges E, de Almeida Ferreira Braga DP, de Sousa Bonetti TC, Iaconelli A, Franco JG (2009) Artificial oocyte activation using calcium ionophore in ICSI cycles with spermatozoa from different sources. Reprod Biomed Online 18:45–52
13. Ebner T, Köster M, Shebl O, Moser M, Van der Ven H, Tews G et al (2012) Application of a ready-to-use calcium ionophore increases rates of fertilization and pregnancy in severe male factor infertility. Fertil Steril 98:1432–1437
14. Du T, Wang Y, Fan Y, Zhang S, Yan Z, Yu W et al (2018) Fertility and neonatal outcomes of embryos achieving blastulation on Day 7: are they of clinical value? Hum Reprod 33:1038–1051
15. Kuang Y, Hong Q, Chen Q, Lyu Q, Ai A, Fu Y et al (2014) Luteal-phase ovarian stimulation is feasible for producing competent oocytes in women undergoing in vitro fertilization/intracytoplasmic sperm injection treatment, with optimal pregnancy outcomes in frozen–thawed embryo transfer cycles. Fertil Steril 101:105–111
16. Chen H, Wang Y, Lyu Q, Ai A, Fu Y, Tian H et al (2015) Comparison of live-birth defects after luteal-phase ovarian stimulation vs. conventional ovarian stimulation for in vitro fertilization and vitrified embryo transfer cycles. Fertil Steril 103:1194–1201
17. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology (2011) The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod 26:1270–1283
18. Gardner DK, Schoolcraft WB (1999) In vitro culture of human blastocysts. In: Jansen R, Mortimer D (eds) Toward reproductive certainty: fertility and genetics beyond 1999. Parthenon Publishing, London, pp 378–388
19. Montjean D, Pauly V, Gervoise-Boyer M, Amar-Hoffet A, Geoffroy-Siraudin C, Boyer P (2019) Is it worth it to cryopreserve embryos with blastulation delay at day 5? Zygote 28:1–6
20. Nasr-Esfahani MH, Razavi S, Javdan Z, Tavalaee M (2008) Artificial oocyte activation in severe teratozoospermia undergoing intracytoplasmic sperm injection. Fertil Steril 90:2231–2237
21. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K et al (2009) International committee for monitoring assisted reproductive technology (ICMART) and the world health organization (WHO) revised glossary of ART terminology, 2009. Fertil Steril 92:1520–1524
22. Zhu Q, Wang N, Wang B, Wang Y, Kuang Y (2018) The risk of birth defects among children born after vitrified blastocyst transfers and those born after fresh and vitrified cleavage-stage embryo transfers. Arch Gynecol Obstet 298:833–840
23. Zhu J, Zhu Q, Wang Y, Wang B, Lyu Q, Kuang Y (2019) Comparative study on risk for birth defects among infants after in vitro fertilization and intracytoplasmic sperm injection. Syst Biol Reprod Med 65:54–60
24. World Health Organization. International statistical classification of diseases and related health problems. Available at: https://apps.who.int/iris/bitstream/10665/61362/1/WHO_MNH_MEP_87.1_REV.2.pdf. Last accessed 17 March 2015
25. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S (2011) Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen–thawed embryo transfer in normal responders. Fertil Steril 96:344–348
26. Cobo A, de los Santos MJ, Castello D, Gamiz P, Campos P, Remohí J (2012) Outcomes of vitrified early cleavage-stage and blastocyst-stage embryos in a cryopreservation program: evaluation of 3150 warming cycles. Fertil Steril 98:1138–1146
27. Wong KM, van Wely M, Mol F, Repping S, Mastenbroek S (2017) Fresh versus frozen embryo transfers in assisted reproduction. Cochrane Database Syst Rev 28(CD011184):27
28. Chen ZJ, Shi Y, Sun Y, Zhang B, Liang X, Cao Y et al (2016) Fresh versus frozen embryos for infertility in the polycystic ovary syndrome. N Engl J Med 375:523–533

29. Miller N, Biron-Shental T, Sukenik-Halevy R, Klement AH, Sharony R, Berkovitz A (2016) Oocyte activation by calcium ionophore and congenital birth defects: a retrospective cohort study. Fertil Steril 106:590–596
30. Wen J, Jiang J, Ding C, Dai J, Liu Y, Xia Y et al (2012) Birth defects in children conceived by in vitro fertilization and intracytoplasmic sperm injection: a meta-analysis. Fertil Steril 97:1331–1337
31. Ooki S (2015) Birth defects after assisted reproductive technology according to the method of treatment in Japan: nationwide data between 2004 and 2012. Environ Health Prev Med 20:460–465
32. Wang N, Lin J, Zhu Q, Fan Y, Wang Y, Fu Y et al (2018) Comparison of neonatal outcomes and live-birth defects after progestin-primed ovarian stimulation versus conventional ovarian stimulation for in vitro fertilization: a large retrospective cohort study. Medicine (Baltimore) 97:e11906
33. Samadirad B, Khamnian Z, Hosseini MB, Dastgiri S (2012) Congenital anomalies and termination of pregnancy in Iran. J Pregnancy 2012:574513
34. Deng C, Yi L, Mu Y, Zhu J, Qin Y, Fan X, Wang Y, Li Q (2015) Dai L Recent trends in the birth prevalence of down syndrome in China: impact of prenatal diagnosis and subsequent terminations. Prenat Diagn 35(4):311–318
35. Hollier LM, Leveno KJ, Kelly MA, Mcintire DD, Cunningham FG (2000) Maternal age and malformations in singleton births. Obstet Gynecol 96:701–706
36. Nasr-Esfahani MH, Deemeh MR, Tavalaee M (2010) Artificial oocyte activation and intracytoplasmic sperm injection. Fertil Steril 94:520–526
37. Nikiforaki D, Vanden Meerschaut F, de Roo C, Lu Y, Ferrer-Buitrago M, de Sutter P et al (2016) Effect of two assisted oocyte activation protocols used to overcome fertilization failure on the activation potential and calcium releasing pattern. Fertil Steril 105:798–806
38. Isachenko E, Isachenko V, Todorov P, Ostashko V, Kreienberg R, Kaufmann M et al (2010) Pregnancy after the calcium ionophore correction of pronuclei position in oocytes after intracytoplasmic sperm injection. Fertil Steril 94(2770):e3–5
39. Whittaker W, Anselmi L, Kristensen SR, Lau YS, Bailey S, Bower P et al (2016) Associations between extending access to primary care and emergency department visits: a difference-in-differences analysis. PLoS Med 13:e1002113