Original Article

Efficacy of Artificial Oocyte Activation in Improving the Reproductive Outcome in Poor Responders: A Single Centre Cohort Study

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Abstract

Objective: Achieving pregnancy in poor ovarian response patients is a challenge. Failed fertilization after ICSI, despite normal semen parameters is due to defects in oocyte activation. In-vitro activation of oocytes using Ca^{+2} agents can be useful in increasing the fertilization rates in these patients. This study aimed to evaluate the efficacy of artificial oocyte activation by calcium ionophores in poor responders in improving fertilization, cleavage, implantation and clinical pregnancy rates.

Materials and methods: This is a prospective, cohort study conducted on 120 patients having poor ovarian response, (POSEIDON criteria) undergoing in-vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) treatment at Southend Fertility and IVF, New Delhi from 1st August 2019 to 31st March 2020. Exclusion criterion was patients with partners with abnormal semen parameters. After OPU patients were randomized into two groups, study group (n=50) underwent ICSI-AOA (ICSI followed by artificial oocyte activation) using calcium ionophore- GM508 Cult-Active Solution) while the controls (n=57) were subjected to ICSI only.

Results: Comparison of ICSI-AOA and ICSI groups showed: (i) number of fertilized oocytes - 2.42 vs. 2.16, p = 0.049 (ii) No. of cleavage stage embryos 2.32 vs. 1.96, p = 0.008 (iii) No. of grade A embryos 1.52 vs. 1.04, p = 0.009 (iv) fertilization rate - 89.00% vs. 83.04%, p = 0.093 (v) cleavage rate - 96.33% vs. 92.55%, p = 0.165 (vi) implantation rate - 27.14% vs. 11.74%, p = 0.098 (vii) clinical pregnancy rate - 34.3% vs. 20.5%, p = 0.167.

Conclusion: The number of fertilized oocytes, grade A embryos and cleavage stage embryos formed after ICSI-AOA were statistically significantly more than ICSI. ICSI-AOA has not shown improvement in fertilization, cleavage, implantation and clinical pregnancy rate. From the present study the conclusive evidence cannot be drawn due to small sample size hence further studies are needed on a larger population.

Keywords: Calcium Ionophore; Poseidon Criteria; Bologna Classification; Intra cytoplasmic Sperm Injection (ICSI)

Introduction

Assisted reproductive technology has made major advances in achieving successful pregnancy in infertile couples, but treating patients with poor ovarian response is still a challenge. Ovarian response measured by number of oocytes retrieved after maximal stimulation is a major predictor of successful cycle (1, 2). Lesser the number of oocytes,

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lesser are the good quality embryos formed, consequently lower pregnancy rates as compared to the normoresponders (3, 4). Thus the outcome of an ART cycle depends upon a number of independent factors including couple's age especially female partner, duration of infertility, response of the ovaries to stimulation, number of oocytes obtained and semen factors to name a few (5, 6). Further, irrespective of the chronological age, women with lesser number of oocytes have higher total fertilization failure rate (TFF) (7).

Many attempts have been made to identify and define poor responders, e.g. Bologna criteria, but proved inadequate in many studies. Recently a new stratification of poor and hypo responders has been defined i.e. POSEIDON stratification. POSEIDON criteria defines, retrieval of < 4 oocytes as poor ovarian response and between 4-9 oocyte retrieval as a hypo response. Diminished ovarian response is mainly seen in advanced age women (>35 years), wherein ovarian reserve falls due to increasing follicle atresia and reduced sensitivity of follicles to FSH (8, 9). But nevertheless patients with good ovarian reserve can also exhibit suboptimal response which may be due to suboptimal gonadotropin dose or genetic polymorphisms that affects endogenous gonadotropins or their receptors (10, 11).

Amongst the different therapeutic strategies adopted to improve the success rate in poor responders ICSI is a major breakthrough, but failed fertilization with ICSI does occur despite of normal semen parameters. This is due to oocyte related deficiencies i.e. defects in oocyte activation (12, 13). As compared to normal fertilization many steps of sperm-oocyte interaction are bypassed in ICSI. The first calcium oscillations begin after a delay of approximately 30 minutes to several hours. This delay is due to the time required by the oocyte's cortical cytoskeleton for breaking the plasma membrane of sperm, removal of acrosome and to expose the sperm factor (PLC zeta) to the oocyte cytoplasm. In this process, by the time calcium oscillations begin; the oocyte might get older (as regards the length of time from LH surge), decreasing its reproductive potential. Sperm chromatin decondensation is also delayed causing failure of fertilization (14, 15).

Oocyte activation defects resulting in failed ICSI can be treated by in-vitro artificial oocyte activation, where intracellular Ca^{+2} oscillations are triggered by enhancing oollemma permeability and by modulating extracellular content to favor calcium influx (16). Three different techniques have been developed to achieve oocyte activation: mechanical, electrical and chemical (17). Chemical oocyte activation is the most accepted technique; involving exposure of ICSI-oocytes to one of the compounds i.e. calcium ionophore A213187, ionomycin, strontium chloride, ethanol, puromycin, phorbol ester and thimerosal. These compounds generate pores in the oollemma causing extracellular calcium ion influx (16, 17, 18, 19).

Recent studies including a randomized controlled trial (20) have studied the role of artificial oocyte activation after ICSI (ICSI-AOA) using calcium ionophore A213187 in improving the fertilization, implantation and pregnancy rate in women with poor ovarian reserve. Although the data is limited, there is no evidence suggesting association of AOA with chromosomal anomalies in the embryos, perinatal morbidity and mortality.

Materials and methods

The present study was a prospective, single center cohort study conducted on a total of 120 women diagnosed with poor ovarian response, as per the POSEIDON criteria (< 4 oocytes retrieved after ovarian stimulation), undergoing treatment at Southend Fertility and IVF, New Delhi from 1st August 2019 to 31st March 2020.

Inclusion criteria

- Patients with poor ovarian response [less than 4 oocytes retrieved at the time of oocyte pick up (OPU)] in the previous as well as present cycle.

Exclusion criteria

- Patients with abnormal semen parameters in male partner. Semen parameters will be assessed as per WHO 2010 criteria.
- Patients in whom more than 4 oocytes were retrieved.

All eligible patients were enrolled for the study after an informed written consent. Complete his-tory was taken and physical examination was done. Detailed demographic data and investigation record including pre-IVF tests, ovarian reserve tests i.e. serum AMH levels, Day 2 S. FSH, LH, Estradiol, Progesterone were noted. All the patients were subjected to pelvic ultrasound to assess antral follicle count (AFC). Ovarian stimulation protocol was individualised depending on the above findings. Oocyte retrieval was planned after 34 to 36 hours of recombinant hCG trigger. Trans-vaginal ultrasound

guided oocyte retrieval was done under general anaesthesia. Patients were randomized using block randomization with blocks of 10 into two equal groups i.e. study and control (n=60 each). ICSI procedure was done in both the groups. Study group patients (n=60) were subjected to artificial oocyte activation (AOA) using calcium ionophores (CI) after ICSI while control group (n=60) women underwent ICSI only. Artificial oocyte activation was done using Calcium ionophore – GM508 Cult-Active solution. The CI solution was incubated in 5.0%- 7.0% CO2 at 37°C for four hours prior to use. After ICSI, MII oocytes were incubated in 50µl calcium ionophore solution for fifteen minutes. Oocytes were later washed with 500µl cul-ture media followed by incubation until fertilization was achieved. Fertilization was confirmed by appearance of two pronuclei. Further, embryo grading was done according to Istanbul consensus 2011 for scoring of embryos and the embryos were graded as "A", "B" and "C". Data was col-lected and compared for stimulation protocol, total dose and duration of gonadotropin used, en-dometrial thickness on the day of hCG trigger, number of total oocyte retrieved, number of MII oocyte obtained, fertilization and cleavage rate, number and grade of embryos, number of embry-os transferred per patient. Following Embryo Transfer (ET), progesterone was given for luteal phase support. Serum b-hCG level was done for all patients on day 14 of embryo transfer. Lev-els >50 ng/ml were suggestive of pregnancy. Implantation rate was calculated as "number of G-sac seen on TVS per number of embryos transferred" and clinical pregnancy rate calculation "was done on the basis of presence of foetal cardiac activity on TVS". Implantation and clinical preg-nancy rate were compared in the two groups. Embryo transfer procedure was cancelled in patients with high P4 values (>1.5ng/ml) at the time of trigger, too thin/ thick endometrium, total fertiliza-tion failure. The observation and outcome was recorded in a performa for each individual case.

Data were analyzed using SPSS version 20. Descriptive statistics were used in the form of Mean/ Standard deviation and medians for continuous variables and percentages for categorical variables. Chi-square test was used for group comparisons for categorical data. Fisher's exact test was used in cases where the expected frequency in the contingency tables was found to be < 5% for > 25% of the cells. For non-normally distributed data Wilcoxon test was used.

Results

A total of 120 women (60 in each group) were initially enrolled for the study. After optimal controlled ovarian hyperstimulation, at the time of oocyte retrieval, 04 patients had empty follicles, 09 patients had only the immature follicles. These patients (n=13) were dropped out from the study. After the final recruitment, 50 patients were there in the study group and 57 patients were in the control group.

Table 1 shows the median age of women in both the groups was 34 years (range 26-44 years). 46.0% of women taken as cases and 47.4% of controls had advanced age (age ≥35 years). Majority of the patients had primary infertility (72% vs. 73.7%) with the mean duration of infertility 2.01 years in cases and 1.72 years in controls. The various causes of infertility in the study and control group were: (i) advanced maternal age (≥35 years) (ii) Tubal Factor (iii) Endometriosis (iv) history of endometrial tuberculosis in the past, (v) Hypothyroidism (vi) More than one above-mentioned factors causing infertility. As shown in table 2 the baseline hormone levels on Day2 of periods which were comparable in the two groups. Mean antral follicle count on Day2 was 5.34 and 5.11 in the cases and controls respectively. Majority of the patients had good ovarian reserve (60% vs. 51%) and belonged to Poseidon group I (40.0% cases vs. 31.6% controls) and Poseidon group II (20.0% cases vs. 19.3% controls). Table 2 shows that maximum number of patients in the two groups, 90% vs. 80.7% respectively received the antagonist protocol for ovarian stimulation. The mean duration of stimulation and total gonadotropin dose required for stimulation was comparable in the two groups. All the patients were given recombinant hCG (rHCG) as trigger. At the time of ovum pick-up the average number of oocytes obtained in the study group were 2.82 and 2.70 in the controls. Table 3 shows that the mean M-II oocytes in the two groups were 2.72 (0.50) and 2.65 (0.55) respectively. The mean number of oocytes fertilized after ICSI- AOA were 2.42 (0.64) and 2.16 (0.70) after ICSI. The average number of cleavage stage embryos formed in the interventional group was 2.32 (0.65) and control group was 1.96 (0.68) ($p = 0.008$). As per the Istanbul consensus of embryo grading, Grade A embryos were significantly more in ICSI-AOA than ICSI group [1.52 (0.95) vs. 1.04 (0.93), $p = 0.009$].

Table 1: Comparison of the two groups on the basis of demographic profile

Parameters	Groups		P value
	Case (n = 50)	Control (n = 57)	
Age (Years)	34.58 ± 3.83	33.89 ± 4.29	0.385[1]
Age			0.887[2]
<35 Years	27 (54.0%)	30 (52.6%)	
≥35 Years	23 (46.0%)	27 (47.4%)	
Husband's Age (Years)	36.04 ± 3.72	35.39 ± 4.34	0.313[3]
Type Of Infertility			0.845[2]
Primary	36 (72.0%)	42 (73.7%)	
Secondary	14 (28.0%)	15 (26.3%)	
Duration Of Infertility (Years)	2.01 ± 1.01	1.72 ± 0.79	0.103[1]
Cause of Infertility			
Advanced maternal age (> 35 years)	23 (46.0%)	27 (47.4%)	0.887
Tubal factors	10 (20.0%)	12 (21.1%)	0.893
Endometriosis	6 (12%)	7 (12.3%)	0.965
H/O endometrial TB in past	3 (6%)	3 (5.3%)	1.000
Hypothyroidism	2 (4%)	6 (10.5%)	0.279
More than one above mentioned causes	37 (74%)	42 (73.8%)	0.965
Poseidon Group			0.649[2]
I	20 (40.0%)	18 (31.6%)	
II	10 (20.0%)	11 (19.3%)	
III	7 (14.0%)	13 (22.8%)	
IV	13 (26.0%)	15 (26.3%)	

1: t-test, 2: Chi-Squared Test, 3: Wilcoxon Test

The average number of blastocysts formed in these patients were 1.25 (0.46) vs. 1.00 (0.00). The mean blastocyst formation rate was 47.92% (cases) and 44.44% (controls).

Majority of the women underwent fresh embryo transfer; 60.0% cases and 71.4% controls while frozen embryo transfer was performed in 10.0% cases and 7.1% controls.

Table 2: Comparison of the two groups on the basis of details of controlled ovarian hyperstimulation

Parameters	Groups		P value
	Case (n = 50)	Control (n = 57)	
Hormonal Levels on Day 2			0.492[4]
S. FSH (mIU/mL)	8.06 ± 2.50	7.74 ± 2.13	0.473[1]
S. LH (mIU/mL)	4.21 ± 3.81	3.38 ± 1.62	0.273[3]
S. Estradiol (pg/mL)	30.07 ± 13.25	34.55 ± 14.00	0.095[3]
S. Progesterone (ng/mL)	0.56 ± 0.61	0.64 ± 0.62	0.188[3]
S. AMH (ng/mL)	1.67 ± 0.91	1.39 ± 0.62	0.085[3]
Day 2 Antral Follicle Count	5.34 ± 1.57	5.11 ± 1.54	0.425[3]
Stimulation Protocol			
AACEP	1 (2.0%)	2 (3.5%)	
Antagonist	45 (90.0%)	46 (80.7%)	
Long Protocol	02 (4.0%)	03 (5.3%)	
Minimal Stimulation	1 (2.0%)	5 (8.8%)	
Stop Protocol	1 (2.0%)	1 (1.8%)	
Total Days of Stimulation	11.20 ± 2.02	11.65 ± 2.06	0.158[3]
Total Gonadotropin Dose	3305.70 ± 1244.46	3319.59 ± 834.16	0.310[3]
Trigger (rHCG)	50 (100.0%)	57 (100.0%)	1.000[2]
Hormonal Levels at the Time of Trigger			0.153[4]
S. Estradiol (pg/ml)	963.98 ± 284.44	1100.25 ± 284.44	0.030[3]
S. LH (mIU/ml)	1.68 ± 1.03	2.15 ± 1.13	0.028[3]
S. Progesterone (ng/ml)	0.75 ± 0.52	0.84 ± 0.50	0.178[3]

1: t-test, 2: Chi-Squared Test, 3: Wilcoxon Test, 4: Fisher's Exact Test

Table 3: Comparison of the two groups on the basis of ICSI-AOA and ICSI outcome

Parameters	Groups		P value
	Case (n = 50)	Control (n = 57)	
Number of M-II Oocytes	2.72 ± 0.50	2.65 ± 0.55	0.512[3]
Number of Fertilized Oocytes**	2.42 ± 0.64	2.16 ± 0.70	0.049[3]
Number of cleavage stage embryos	2.32 ± 0.65	1.96 ± 0.68	0.008
Number of Grade A Embryos**	1.52 ± 0.95	1.04 ± 0.93	0.009[3]
Number of Grade B Embryos	0.70 ± 0.79	0.82 ± 0.81	0.434[3]
Number of Grade C Embryos	0.10 ± 0.30	0.14 ± 0.35	0.507[3]
Number of Blastocysts	(n=8) 1.25± 0.46	(n=3) 1.00 ± 0.00	0.447[3]
Blastocyst formation rate (%)	47.92 ± 24.30	44.44 ± 9.62	0.822
Type of Embryo Transfer			0.505[4]
Fresh	30 (60.0%)	40 (71.4%)	
Frozen (FET)	5 (10.0%)	4 (7.1%)	
No embryo transfer / Vitrification	15 (30.0%)	12 (21.4%)	
Number of Embryos Transferred	2.14 ± 0.65	2.00 ± 0.57	0.289[3]
Day of Embryo Transfer	(n=35)	(n=44)	0.114[4]
Day 2	1 (2.9%)	7 (15.9%)	
Day 3	27 (77.10%)	33 (75.0%)	
Day 5	3 (8.6%)	3 (6.8%)	
DAY 3, 5	4 (11.4%)	1 (2.3%)	
Fertilization Rate (%)	89.00 ± 17.37	83.04 ± 18.93	0.093[3]
Cleavage Rate (%)	96.33 ± 11.33	92.55 ± 15.56	0.165[3]
Implantation Rate (%)	27.14 ± 41.04	11.74 ± 26.06	0.110[3]
Clinical Pregnancy Rate (Positive)	12 (34.2%)	9 (20.45%)	0.167[2]

**Significant at p<0.05, 1: t-test, 2: Chi-Squared Test, 3: Wilcoxon Test, 4: Fisher's Exact Test

The mean number of embryos transferred in the two groups was comparable: 2.14 (0.65) vs. 2.00 (0.57). Majority of the patients underwent Day 3 embryo transfer: 77.1% vs. 75% cases and controls respectively. The mean fertilization rate in the case and controls was 89.00 (17.37%) vs. 83.04 (18.93%) respectively (p = 0.093). The mean cleavage rate in the two groups was 96.33 (11.33%) vs. 92.55 (15.56%) (p = 0.165). The mean implantation rate in the ICSI-AOA group and ICSI group was 27.14 (41.04%) vs.11.74 (26.06%). Clinical pregnancy was detected in 34.3% women who underwent ICSI-AOA as compared to 20.5% in ICSI group (X^2 = 1.911, p = 0.167).

Discussion

In most of the studies on poor responders, these patients are recruited on the basis of exhibition of poor ovarian response in previous ovarian hyperstimulation cycles and/or tests suggesting poor ovarian reserve (21). In the present study, initial enrollment of poor responders was based on the same criteria. After COH with adequate gonadotropin dose the patients still exhibiting poor ovarian response (< 4 oocytes) on the day of OPU were finally recruited for the present study, thus removing the selection bias.

In an attempt to increase FORT (follicular output rate) and FOI (follicular oocyte index) in poor responders various strategies have been documented i.e. use of recombinant-FSH with increased rFSH dose, rLH supplementation, DHEAS use before OS, dual stimulation. None of the trials as yet has explicitly evaluated the effectiveness of these interventions (22). Based on these findings we speculated that this set of patients might benefit from oocyte activation, as this intervention is independent of the stimulation protocol chosen. The real focus should be to obtain the best results out of 'limited number of oocytes available' in both expected and unexpected poor responders.

In the present study iCOS (individualised controlled ovarian stimulation) was given to optimize the results. Antagonist protocol (23- 25) was given to majority of our patients (90% cases vs. 80.7% controls). Long protocol was adopted for cyst suppression in patients with Persistent corpus luteal cyst (4% cases and 5.3% controls). A meta-analysis

including 17 RCTs showed **"**no significant difference in the number of oocytes retrieved (mean difference 0.09; 95% CI 0.53-0.36) and clinical pregnancy rate (OR- 1.24, 95% CI 0.88-1.73) between the GnRH long agonist and antagonist protocol in poor responders (26).

In a few selected patients i.e. advanced age group with poor ovarian reserve and previous multiple failed IVF/ICSI cycles using antagonist protocol; AACEP protocol Fisch et al. (27) (Gonadotropin releasing hormone agonist/antagonist conversion with estrogen priming; 2% cases vs. 3.5% controls) and minimal stimulation protocol Mitwally and Casper (28) Goswami SK et al. (29). (Letrozole in combination with gonadotropins; 2% cases vs. 8.8% controls) was given. Minimal stimulation protocol was adopted as a last resort before offering donor oocytes to the poor responders.

Despite employing different stimulation protocols, the peak E_2 levels at the time of trigger (963.98 ± 284.44 pg/ml vs. 1100.25 ± 284.44pg/ml) were comparable in the two groups. Mean M II oocytes obtained were also comparable (2.72 ± 0.50 vs. 2.65 ± 0.55) but ICSI followed by artificial oocyte activation achieved higher number of fertilized oocytes as compared to ICSI only group (2.42 ± 0.64 vs. 2.16 ± 0.70, p-0.049) showing statistically significant difference. The above findings indicate that, the calcium ionophore used in ICSI-AOA increases calcium concentration in the cytoplasm enabling its activation resulting in successful fertilization. The total fertilization rate with ICSI-AOA was higher than ICSI (89.00 ± 17.37 vs. 83.04 ± 18.93, p = 0.093). Although this difference was not statistically significant, the results indicate that ICSI-AOA improves fertilization rate in poor responders.

Another advantage of ICSI-AOA in the present study was that, none of the patients in ICSI-AOA group had total fertilization failure as compared to ICSI where total fertilization failure rate was 1.75%. Khamsi et al. documented that 2.9% women had total fertilization failure after ICSI despite normal sperm parameters (30). Studieshave been reported demonstrating better fertilization rates with calcium ionophores in couples who have experienced fertilization failures in previous ICSI cycles regardless of the male factor (31- 33).

Ca^{+2} signalling following oocyte activation plays a pivotal role in pronuclear union, in initiating first and subsequent embryonic cleavages (34, 35). In the present study the cleavage rates (96.33% ± 11.33 vs. 92.55% ± 15.56, p = 0.165), the number of embryos formed at day 2 check (2.32 ± 0.65 vs. 1.96 ± 0.68, p = 0.008), the number of cleavage stage grade A embryos (1.52 ± 0.95 vs. 1.04 ± 0.93, p = 0.009) were higher in oocyte activation group in comparison to ICSI group. Although some of the findings do not show statistically significant difference but these observations suggest the beneficial role of calcium signalling in development of patterning and morphogenesis of early embryos as demonstrated by Whittaker (36) and Ebner et al. (37).

In the present study, we preferred to transfer the embryos at the cleavage stage so as to prevent the embryo loss in the extended cultures. Nonetheless, blastocyst transfer was performed in patients (n=11, 13.9%) with certain indications i.e. previous implantation failures in cleavage stage embryo transfer, PGS testing, and those who insisted on blastocyst transfer only. Ebner et al. (37) found a significant improvement in blastocyst formation rate (47.6% vs. 5.5%) after artificial oocyte activation in patients with complete developmental arrest/delay or poor blastocyst formation rate (<15%) in their previous cycles. However, in the present study the blastocyst formation rate was comparable in the two groups (47.92 ± 24.30% vs. 44.44 ± 9.62%). The probable explanation for the insignificant difference could be that in ICSI-AOA group embryos cultured till blastocyst stage were from relatively advanced age women as compared to the ICSI only group (38 years vs. 33.66 years). Although AOA had improved the blastocyst formation rate but it could not compensate for "the age related abbrations in the oocyte quality", thus affecting the blastocyst formation capacity. The small sample size also affected the results.

Conflicting evidence is available in the literature with regards to "the perfect timing for embryo transfer" and the "optimal embryo transfer strategy" in poor responders. In the present series majority of our patients had undergone fresh embryo transfer in both the groups (60% vs. 71.4%). Fresh transfer was deferred in the patients with high P4 values (>1.5ng/ml) at the time of trigger, where PGS testing was needed, too thin/thick endometrium at the time of trigger.

Day 3 embryo transfer was preferred in most of the women of the two groups (77.1% vs. 75%) as day 3 transfer allows expression of additional morphological features that increases the chances of successful implantation (38). On the contrary,

Bahceci et al in a RCT concluded that exposing the embryos to in-vitro conditions for an extended time (till day 3) might hamper the developmental competency and there might occur embryo loss. In the present study 3% (n=1) cases and 16% (n=7) controls had embryo developmental arrest in previous cycles and in present cycle low-grade embryos were formed, day 2 transfer was chosen, considering the results of Bahceci et al. (39).

On the basis of Cochrane review (40) of 12 RCTs favoring blastocyst transfer, Berkkanoglu et al. (41) had attempted blastocyst transfer in poor responders as well, achieving encouraging results. Despite the above knowledge, in the present study blastocyst transfer was performed in only 13.9% women that too with specific indications so as to avoid embryo loss in extended cultures.

In few of our patients (6.3%), dual transfer i.e. day3 and day 5 transfer was also performed. These patients had specifically demanded day 5 transfer but because previously they had embryo loss in extended cultures, we counseled them for dual transfer so as to prevent this risk. Fortunately, all these patients had successful blastocyst formation.

Embryos were cryopreserved in 25% (n=27) patients for the following reasons: 25% (n=7) of them wanted embryo collection prior to transfer. 15% (n=4) of these women had postponed ET due to personal reasons. In the remaining patients (60%, n=16), embryo transfer had to be postponed due nationwide lockdown/curfew because of COVID-19 pandemic (42).

Secondary outcome in the present study was clinical pregnancy rate and implantation rate. "Clinical pregnancy rate per cycle" (34.3% vs. 20.5%, p = 0.167, odds ratio 2.03, 95% CI = 0.81-3.49) and the "implantation rate per embryo transfer" (27.14 ± 41.04 vs. 11.74 ± 26.06, p = 0.098) was higher in ICSI-AOA group in comparison to ICSI patients although differences were not statistically significance.

In subgroup analysis unexpected poor responders had significantly higher clinical pregnancy rate with calcium ionophore use as compared to the patients where CI was not used (41.66% vs. 23.80%, p = 0.046). The clinical pregnancy rate in expected poor responders was comparable in two groups (18.18% vs. 17.39%) indicating that calcium ionophore was not beneficial in this class of patients with regards to clinical pregnancy rate and further studies are needed to arrive at a conclusion. Aytac et al. (20) demonstrated no significant difference in the clinical pregnancy rate in expected poor responders with calcium ionophore use (13.6% vs. 8.1%, p = 0.13).

Meta-analysis of 14 studies (43) to demonstrate the effect of artificial oocyte activation concluded that overall pregnancy rate (per ET; 36.9% vs. 15.5%, OR-3.48; 95% CI 1.65-7.37) and live birth rate (26.8% vs. 9.8%, OR 3.33; 95% CI, 1.50-7.39) increased with the use of calcium ionophores. It was further demonstrated that statistically significant improvement in the fertilization rate (59.2% vs. 47.4%, OR 3.74, 95% CI= 1.84-7.57), cleavage rate (68.3% vs. 48.7%, OR 2.28, 95% CI= 1.23-4.21) and blastocyst formation rate (51% vs. 10.7%, OR 6.70, 95% CI= 2.59-17.28) was noted with the use of calcium ionophores for AOA. This meta-analysis has included the studies using AOA for various indications (previous TFF/LF, embryo development arrest/ delay, decreased blastulation rate, diminished ovarian reserve).

Limitations

- Small sample size and heterogeneity in patient population.
- For an ideal comparison sibling oocytes from each women should have been split into two halves and subjected to ICSI-AOA and ICSI respectively. But this was not practically feasible for us due to poor yield of oocytes.
- Use of different stimulation protocols, different timings and strategies of embryo transfer, which was inevitable.

Conclusion

The number of fertilized oocytes, grade A embryos and cleavage stage embryos formed after ICSI-AOA were statistically significantly more than ICSI. Thus, artificial oocyte activation acts at the next step after ICSI i.e. it improves the cytoplasmic competency of the available oocytes further enhancing the fertilization capacity of the gametes and better embryo development. ICSI-AOA has not shown improvement in fertilization, cleavage, implantation and clinical pregnancy rate. To the best of our knowledge, the present series is the first prospective study evaluating the efficacy of calcium ionophores in poor responders. Although a conclusive evidence cannot be drawn due to small sample size, further studies are needed on a larger population.

Conflict of Interests

There are no conflicts of interest among the authors.

Acknowledgments

The present study has been approved by the appropriate ethical committee and has been performed in accordance with the ethical standards as laid down in 1964 Declaration of Helsinki.

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