Original Article

Impact of modified intracytoplasmic sperm injection technique on *in vitro* fertilization outcomes

Rohit Bansal, Gaurav Majumdar, Abha Majumdar, Puneet Singh Lall

Center of IVF and Human Reproduction, Sir Ganga Ram Hospital, New Delhi, India

Abstract

The objective of the study was to evaluate the impact of modified intracytoplasmic sperm injection (ICSI) technique on *in vitro* fertilization outcomes. The study was conducted in a tertiary infertility center, New Delhi. In this retrospective cohort study, we retrospectively analyzed 247 ICSI cycles. Different inclusion and exclusion criteria were applied to remove the biasness of study. The 247 ICSI cycles were divided into two groups. Group A ($n = 152$) in which the patient underwent through modified ICSI technique), Group B ($n = 95$) in which patient underwent conventional ICSI). Modified technique involves repeated in and out movements of the injection pipette to break the oolemma without applying negative pressure to reduce the detrimental effect on oocyte. Fertilization rate was found to be higher in modified ICSI group when compared with conventional ICSI (70% vs. 63%; $P = 0.0006$). The embryo utilization rate was found to be similar in both the groups (38.4% vs. 40.2%). The overall blastocyst utilization rate was also comparable between both the groups. The mechanical modification of not applying negative pressure during ICSI resulted in a significant increase in fertilization rate. A larger study is required to validate the technique in terms of other embryologic parameters.

Keywords: Blastocyst utilization rate, conventional ICSI, embryo utilization rate, fertilization rate, modified ICSI

Address for correspondence: Dr Gaurav Majumdar, Center of IVF and Human Reproduction, Sir Ganga Ram Hospital, New Delhi 110060, India.
E-mail: gaurav1979@hotmail.com
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INTRODUCTION

Infertility is a global health issue affecting millions of people of reproductive age worldwide. Around 48 million couples and 186 million individuals have infertility globally.[1] Available data suggest that 8% to 12% of couples suffer from infertility and infertility rates vary dramatically between countries and regions. In India, the burden of infertility among couples ranged from 4% to 17%.[2] Infertility may occur due to male factors, female factors, a combination of male and female factors, or maybe unexplained.[3]

Intracytoplasmic sperm injection (ICSI) is one of the most promising methods in assisted reproductive technology for a patient with suboptimal semen parameters such as oligoasthenoteratozoospermia (OAT) or in couples who experience low or total fertilization failure.[4,5] ICSI is also necessary for male patients suffering from obstructive and nonobstructive azoospermia.[6] Other indications for ICSI are preimplantation genetic diagnosis[7] and HIV-discordant couples to minimize the exposure of the oocyte to multiple spermatozoa.[8]. Fertilization rates of up to 65% and clinical pregnancy rates up to 45% have been achieved in ICSI cycles.[9] According to the Vienna

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consensus published in 2017, minimum competency for ICSI is denoted by a normal fertilization rate of 65%, whereas the benchmark value is 80%.[10]

Failure of an oocyte to fertilize after ICSI may be attributed to multiple factors among which the inherent quality of the oocyte and the sperm seem to be the most important. Other than gamete morphology and number, another factor that could be responsible for the failure of fertilization after ICSI includes incorrect sperm injection which may either lead to the expulsion of sperm after injection from the cytoplasm or failed activation of the oocyte.[11] In the past, modifications in injection techniques have been suggested to address and overcome this problem of failed oocyte activation after ICSI.[12] The first such modification in the conventional ICSI technique was reported by Tesarik *et al.* in 2002, which involved repeated dislocation of the central ooplasm to the periphery, thus increasing the intracellular concentration of free calcium either by creating an influx of calcium ions[13] or by the release of calcium stored in cell organelles.[14] The second strategy that was reported, involved the aspiration of ooplasm from the periphery, having a large number of mitochondria with higher inner potential (high metabolic ATP activity) and release to the center of the ooplasm along with spermatozoa[15] in a bid to improve the fertilization rate and blastocyst formation in patients with previous fertilization failure using conventional ICSI.

During conventional ICSI, it is common practice to apply negative pressure to break the oolemma, which results in an influx of a sizeable amount of cytoplasm into the injection pipette. However, the extent of the pressure applied may have a detrimental effect on the developing oocyte. According to the study by Dumoulin *et al.*, blastocyst development is compromised if the volume of ooplasm aspirated is greater than 6 pl.[16] Also, aspiration of ooplasm during ICSI may be associated with cytoskeletal damage in zygotes and may lead to three pronuclie.[17,18] Hence, we sought to introduce a modification in the injection technique which involves breaking the oolemma without applying any negative pressure. This will be carried out via repeated in and out the movement of the injection pipette in the oocyte cytoplasm to break the oocyte membrane while avoiding aspiration of the cytoplasm into the needle. Also, we believe that the proposed modification will ensure that the sperm once injected will not be expelled from the cytoplasm after ICSI.

Thus, this study aims to compare the embryologic outcomes of this modified ICSI with conventional ICSI, and further investigate whether such a mechanical modification in technique could result in improving outcomes such as fertilization rate and embryo development rate.

MATERIALS AND METHODS

A retrospective cohort analysis was conducted during the period between January 2019 and March 2020 at the Centre of IVF & Human Reproduction, Sir Ganga Ram Hospital. In this study, we retrospectively analyzed 247 ICSI cycles. The inclusion criteria were (a) patient age less than 38 years and (b) all patients undergoing *in vitro* fertilization (IVF)-ICSI; and the exclusion criteria were (a) number of retrieved oocytes <6, (b) number of mature oocytes injected <4, (c) frozen-thawed oocytes, and (d) severe male factor including severe OATs, severe oligozoospermia, severe tetrazoospermia, severe asthenozoospermia, as well as testicular and epididymal sperm. The 247 ICSI cycles were divided into two following groups: group A ($n = 152$) in which the patient underwent through modified ICSI technique and group B ($n = 95$) in which patient underwent conventional ICSI. Subsequently, embryologic outcomes were evaluated between the two groups.

Ovarian stimulation

All women undergoing IVF-ICSI was subjected to a standard controlled ovarian stimulation protocol. Oocytes were aspirated 36 hours after human chorionic gonadotropin administration by a transvaginal ultrasonographic procedure. Following follicular puncture, the oocytes were cultured for at least 3 hours in G-IVF media (Vitrolife, sweden) before they were exposed to Hyase-10X (30 seconds; Vitrolife) to facilitate mechanical removal of the cumulus cells. All the morphologic parameters of the oocytes wereevaluated before ICSI. Anomalies related to cytoplasm (dark cytoplasm, refractile bodies, dark incorporations, and vacuoles) and anomalies of the outer layer (fragile oolemma, dark zona pellucida, large perivitelline space, and irregularity in shape) were excluded.

Sperm preparation

Fresh semen ejaculates were prepared by discontinuous density gradient centrifugation (80–40%; Pure Sperm, Nidacon Laboratories, Göteborg, Sweden). After liquefaction at room temperature, 1 ml of the semen sample was placed on a gradient consisting of a 1-ml layer of the 80% fraction at the bottom and a 1-ml layer of the 40% fraction at the top, and centrifuged at 300*g* for 15 minutes. After gradient centrifugation, 200 ml of the

bottom fraction was aspirated and washed twice at 300*g* for 10 minutes. After washing, the concentration and motility of the final fraction were assessed. Where necessary, the fraction was diluted or concentrated. Sperm morphology was assessed using the strict Kruger criteria.[19]

Conventional ICSI

The conventional technique of ICSI has been described in detail[20] In brief, micromanipulation was performed on an inverted microscope (200× magnification; Nikon ECLIPSE TE300, Nikon NARISHIGE) with an electronically controlled heat stage and hydraulic micromanipulators (Nikon NARISHIGE). To perform ICSI, the oocyte was held in place with a holding pipette (Inner diameter: 25 μm; TPC, Mornington, Australia) in a way that the polar body was located either at 6 or 12 o'clock position. Then the sperm was brought near the tip of the injecting pipette and the needle was injected into the zona pellucida at 3 o'clock position. Once the zona was pierced, a small amount of ooplasm was aspirated by applying negative pressure (suction) and then slowly released back into the oocyte. The ooplasm was aspirated and released one more time to make sure that oolemma was properly breached and enough mechanical stimulus was provided to the oocyte for activation before the sperm was successfully placed into the ooplasm.

Modified ICSI

The difference between standard and modified ICSI involved a modification in the injection technique. In modified ICSI, unlike conventional ICSI, the sperm was positioned about a centimeter from the tip of the needle before it is injected into the oocyte. The oocyte was held in place with a holding pipette in a way that the polar body was either at 6 or 12 o'clock position. During injection, repeated in and out movements of the injection pipette without applying any negative pressure were performed to break the oolemma. As no negative pressure was applied, membrane breakage was not marked by cytoplasm rushing inside the injection pipette. As soon as the oolemma was breached, as identified by feel, the sperm was injected into the cytoplasm and the pipette was gently withdrawn from the egg.

Statistical analysis

Statistical analysis was performed using Statistical Package Social System (SPSS, IBM Inc.). Continuous variables were presented as mean ± standard deviation or median for non-normally distributed data. Categorical variables were expressed as frequencies and percentages. The comparison of normally distributed continuous variables between the groups will be compared using Chi-squared test or Fisher exact test as appropriate. Non-normal distribution continuous variables will be using Mann–Whitney *U* test. For all statistical tests, a *P*-value less than 0.05 will be considered as significant.

RESULTS

Table 1 demonstrates the baseline characteristics of the cohort. A total number of 247 ICSI cycles were performed. Out of which, 152 subjects were undergone modified ICSI, whereas 95 subjects were undergone conventional ICSI. The mean age for the study population was 31.39 ± 4.2 years. There was no significant difference with respect to age between modified ICSI and conventional ICSI. The number of oocyte allocated to modified ICSI and conventional ICSI was 9.0 and 8.2, respectively. In modified ICSI and conventional ICSI, 1373 and 780 oocytes were injected out of 1769 and1095 number of oocytes, respectively (77.6% vs. 71.2%; $P < 0.00012$). There was approximately same percentage of oocyte damage in both the techniques as more number of oocytes were injected in modified ICSI (3.5% vs. 3.3%). Fertilization rate was found higher in modified ICSI when compared with conventional ICSI (70.7% vs. 63.4%; $P = 0.0006$). The higher fertilizable embryo yield, the more number of utilizable embryo (32.7% vs. 29.9%).

DISCUSSION

There are number of factors which may attribute to failure of an oocyte to fertilize after ICSI among which are oocyte quality, sperm-borne factors seem to be the most important. Another factor which could be responsible for the failure of fertilization after ICSI includes incorrect sperm injection which may either lead to the expulsion of sperm after injection from the

Table 1: Baseline characteristics of cohort

Parameters	Modified ICSI	Conventional ICSI	*P*-value
Patient number (*N*)	152	95	
Age, years(mean ± SD)	31.7 ± 4.1	30.6 ± 4.3	0.023
No. of oocytes retrieved(mean ± SD)	11.6 ± 5.1	11.5 ± 5.3	0.36
No. of oocytes injected(mean ± SD)	9.0 ± 3.9	8.2 ± 3.7	0.052

ICSI, intracytoplasmic sperm injection; SD, standard deviation.

Table 2: Comparison of fertilization rate between the modified ICSI and conventional ICSI techniques

Parameters	Modified ICSI (n = 152)	Conventional ICSI (n = 95)	P-value
No. of eggs assigned	1769	1095	
No. of eggs injected	1373 (77.6%)	780 (71.2%)	0.000122
No. of 2 PN	970 (70.7%)	495 (63.4%)	0.00058
No. of damaged eggs	49 (3.5%)	26 (3.3%)	NS
No. of day 3 embryos utilized	82 (8.4%)	73 (14%)	0.00417
No. of day 5/6 embryos utilized	291(32.7%)	126 (29.9%)	0.062
Total embryo utilization rate	373 (38.4%)	199 (40.2%)	NS

ICSI, intracytoplasmic sperm injection.

cytoplasm or failed activation of the oocyte.[11] There are some reports which suggested the modifications in injection techniques to overcome this problem of failed oocyte activation after ICSI.[12]Mechanical bypassing of both the zona pellucida and the oolemma may dramatically alter the dynamics of meiotic spindle formation which results into degenerated oocytes.[16,21] It has been postulated that the injection procedure can physically disrupt the cytoplasmic organization of the oocyte. Nagy *et al.* demonstrated that the type of membrane breakage during ICSI can influence oocyte survival and embryo development rates.[22].

Our study shows that our modified technique used to rupture the oolemma during ICSI had a significant impact on fertilization rate. Rupturing the oolemma with repeated in and out movements of the injection pipette in the oocyte cytoplasm without applying negative pressure resulted in approximately 7% statistically significant increase in fertilization rate. The oocyte damage rate was similar in both the techniques despite of fact having greater number of oocytes were injected in modified ICSI. It was further found that increase in rate of fertilization in modified ICSI did not manifest into higher percentage of utilizable blastocyst due to small number [Table 2]. Therefore, modified ICSI was found to be better than conventional ICSI in terms of embryologic outcomes. As shown in our study, slight modification in conventional ICSI improves the fertilization rates as well as utilizable embryos.

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Conflicts of interest

There are no conflicts of interest.

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