Original Article

Are we Justified Doing Routine Intracytoplasmic Sperm Injection in Nonmale Factor Infertility? A Retrospective Study Comparing Reproductive Outcomes between *In vitro* Fertilization and Intracytoplasmic Sperm Injection in Nonmale Factor Infertility

Reeta Biliangady[1], Poornima Kinila[1], Rubina Pandit[1], Nutan Kumari Tudu[2], Uma Maheswari Sundhararaj[1], Indu S. T. Gopal[1], Ambika G. Swamy[2]

Departments of [1]Reproductive Medicine and [2]Embryology, Cloudnine Fertility, Bengaluru, Karnataka, India

Abstract

Introduction: Intracytoplasmic sperm insemination (ICSI) came into use in 1992 to improve fertilization in couples with male factor infertility undergoing in vitro fertilization (IVF) or in couples with fertilization failure in a prior IVF cycle. Our aim was to find out if routine ICSI has any additional benefit over conventional IVF in non male factor cases in modern Assisted Reproductive Technology (ART). **Methods:** This is a retrospective single centre study undertaken at a private IVF center. A total of 350 patients with normal male factor were included in the study of which 186 underwent conventional IVF and 134 were subjected to ICSI. They were then compared for various reproductive parameters with Live Birth Rate (LBR) being the primary outcome. *P* value < 0.05 was considered statistically significant. **Results:** Fertilization rates (89.99% vs 85.1%), Blastocyst formation rates (62.86% vs 50.61%) and clinical pregnancy rates (37.85% vs 32.35%) were found to be higher in the IVF group compared to the ICSI group though not statistically significant. The live birth rates in the IVF group was also higher than the ICSI group (32.71% vs 24.26%). **Conclusion:** IVF edged over ICSI in all aspects resulting in better clinical outcome with higher take home babies in non-male factor infertility. Our results show that routine ICSI should not be used as a blanket therapy for all cases in ART.

Keywords: *IVF, ICSI, nonmale factor infertility*

Introduction

In 1992, the process of intracytoplasmic sperm injection (ICSI) was introduced to overcome fertilization problems in couples with male factor infertility or couples with fertilization failure in a prior *in vitro* fertilization (IVF) cycle.[1-3] In the current scenario, clinicians are using ICSI as a routine in most cycles recruited for IVF. Boulet *et al*. reported an increase of 32% in the use of ICSI over the past 15 years.[4] Furthermore, the use of ICSI for male factor infertility has increased from 15% to 67%. A similar rising trend has been reported by the international committee for monitoring assisted reproductive technologies.[5] The report showed that 65% of IVF cycles in Europe performed ICSI. Despite its increased usage, evidence demonstrating improved reproductive outcomes with routine use of ICSI is still lacking.[6] More randomized controlled trials (RCTs) are needed to derive definite conclusions on advantages of ICSI over conventional IVF in nonmale factor infertility.[7] Our aim was to compare reproductive outcomes (live birth rates [LBRs]) between routine ICSI and IVF in nonmale factor infertility.

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Address for correspondence: Dr. Poornima Kinila, Cloudnine Fertility Center, 16/A, 2nd Floor, 9th Main Road, Jayanagar 3rd Block, Bengaluru - 560 011, Karnataka, India.
E-mail: poornima27in@yahoo.co.uk

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Subjects and Methods

This was a retrospective study performed at a private infertility clinic. Patients' who have undergone IVF/ICSI procedures between January 2012 and December 2017 and who fulfilled the inclusion criteria were included in the study. The data were collected from the medical records maintained at the center. Any additional information was collected from the patients through telephonic conversation.

Inclusion criteria

Woman's age between 25 and 35 years, normal ovarian reserve tests (Day 2 follicle stimulating hormone (FSH) <10 IU/L, anti-Mullerian hormone (AMH) >1.0 ng/ml, antral follicle count (AFC) between 5 and 15), optimal endometrium on the day of transfer (thickness of more than 7 mm, triple-line pattern), good quality blastocysts available for fresh transfer (with at least one A component, i.e., AA, AB, or BA) and normal semen parameters (in accordance with the WHO 2010 criteria i.e., sperm count >15 million/ml, normal morphology ≥4%, and progressive motility of ≥32%).

Exclusion criteria

Advanced maternal age >38 years, body mass index (BMI) >35 kg/m^2, low ovarian reserve (Day 2 FSH >10 IU/L, AMH <1.0 ng/ml, and AFC <5 oocytes), suboptimal endometrium <7 mm or any uterine anomalies affecting implantation (polyp, fibroid, and Asherman's syndrome), male factor infertility, surgically retrieved sperm, third party reproduction, cases where preimplantation genetic diagnosis was done, history of recurrent implantation failure.

The decision regarding the insemination method was made by the patients' respective treating consultant. Our fertility unit includes clinicians who manage patients independently. In our center, few consultants do IVF in cases where there is no male factor thereby reserving ICSI to suboptimal semen parameters while others routine perform ICSI irrespective of male factor. Hence, the patients were segregated into two groups:

- Group I: nonmale factor cases who underwent IVF
- Group II: nonmale factor cases who underwent ICSI.

As per the clinician's decision, the controlled ovarian stimulation (COS) was done with GnRH antagonist protocol. COS was started with a flexible starting dose of recombinant/highly purified FSH (Gonal F – Merck Serono) or highly purified human menopausal gonadotropin (HMG) (Menopur HP – Ferring Pharmaceuticals) ranging from 150 to 300 IU, depending on age, BMI, history of previous cycle response, and results of ovarian reserve tests. The FSH or HMG dose was then adjusted according to follicular growth monitored by transvaginal ultrasound every 2–3 days. After at least one follicle reaches 14 mm diameter, the antagonist Ganirelix as Orgalutron 0.25 mg (Organon India Ltd) was added along with FSH or HMG. When at least two follicles reach a mean diameter of 18 mm, 250 mcg of recombinant human chorionic gonadotropin (r-HCG) (r-HCG Inj Ovitrelle 250 mcg- Merck, Switzerland) was administrated and oocytes retrieval was carried out under ultrasound guidance 34–36 h after HCG injection.

After finishing oocyte retrieval, they were then either treated by conventional IVF (Group I) or by ICSI (Group II) with the processed normozoospermic samples.

In conventional IVF (Group I), the maturity status of the oocytes was not examined until after 16–18 h. The oocyte–cumulus complexes were inseminated with 50,000 motile spermatozoa per insemination dish, containing 3–4 oocytes. After completion of 16–18 h incubation time, all of the inseminated oocytes were stripped from the cumulus cells for checking and recording the fertilization and the maturity status of the unfertilized oocytes (Metaphase II [MII], MI, or germinal vesicle).

For ICSI cycles (Group II), cumulus stripping was performed 2 h after oocyte retrieval to examine oocyte maturation. MII oocytes were inseminated with the partner's spermatozoa using the ICSI technique. ICSI was performed at least 1 h after removing the cumulus cells. Immature oocytes were discarded. Post 16–18 h of insemination, fertilization was assessed by the appearance of two distinct pronuclei and two polar bodies.

The normal fertilization was defined as zygotes with two pronuclei (2PN). Zygotes with 2PN were cultured in single step medium (Vitrolife). Embryonic development was assessed on day 2 and day 3 after insemination. Embryos were graded as good, fair, and poor based on the number of blastomeres, size of blastomeres, and degree of fragmentation. If there were at least three good quality embryos on day 3, then the culture was extended to the blastocyst stage in the same medium, and the ET was done on day 5.[8] Day 5 blastocyst (s) were graded according to Gardner and Schoolcraft grading system.[9,10] Supernumerary good quality embryos were cryopreserved at the blastocyst stage.

One or two good quality blastocysts were transferred on day 5. Luteal phase support was started with micronized natural progesterone 400–800 mg/day (oral/vaginal/transdermal) on the day of oocyte retrieval and was continued till 10 weeks of gestation.

Outcome measures

The primary outcome was LBR defined as the rate of deliveries that resulted in at least one live-born baby per transfer. Secondary outcomes measured were implantation rate (IR), biochemical pregnancy, clinical pregnancy rate (CPR), and abortion rate (AR). IR was defined as the ratio of number of intrauterine gestational sacs detected on ultrasound to the number of transferred embryos. Biochemical pregnancy was defined by positive beta-HCG 12–14 days after embryo transfer. CPR was defined as pregnancy confirmed by ultrasound visualization of the gestational sac with fetal cardiac activity between 6th and 7th weeks of gestation from the last menstrual period. AR was defined as miscarriage occurring before 22 weeks of gestation after confirmation of intrauterine gestational sac on an early ultrasound.

Statistical analysis

Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency and proportion for categorical variables.

All quantitative variables were checked for normal distribution within each category of explanatory variable using visual inspection of histograms and normality Q-Q plots. Shapiro–Wilk test was also conducted to assess normal distribution. Shapiro–Wilk test $P > 0.05$ was considered as the normal distribution. The association between categorical explanatory variables and quantitative outcome was assessed by comparing the mean values. Independent sample *t*-test was used to assess statistical significance.

Categorical outcomes (CPR, miscarriage rate, IR, and LBR) were compared between study groups (IVF vs. ICSI) using Chi-square test.

$P < 0.05$ was considered statistically significant. IBM SPSS version 22 (SPSS software, SPSS Inc., Chicago, IL, USA). was used for statistical analysis.[11]

Results

The demographic information and cycle statistics were compared between the two groups with all other outcomes as outlined in Table 1. A total of 350 patients were included in the study. Of the 350 patients, 214 underwent conventional IVF (Group I) and 136 patients underwent ICSI (Group II). The average age, mean number of oocytes retrieved, serum estradiol, and progesterone levels (on the day of retrieval), and endometrial thickness were comparable between the two groups. There were statistically significant higher numbers of MII oocytes obtained as well as a higher number of embryos available for transfer/freezing in the IVF group when compared to the ICSI group.

In addition, the IVF group had statistically significant higher fertilization rate (89.9% vs. 85.1%, $P < 0.001$), cleavage rate (94.4% vs. 92.7%, $P = 0.0001$), and the blastocyst formation rate (62.86% vs. 50.61%, $P < 0.001$) compared with the ICSI group [Figure 1].

The live birth rates (32.71% vs. 24.26%, $P = 0.09$), clinical pregnancy rates (37.85% vs. 32.35%, $P = 0.29$), and implantation rates (101/368, 27.45% vs. 52/233, 22.32%, $P = 0.159$) [Figure 2] were higher in the IVF group as compared to the ICSI group; however, the difference was not statistically significant. The abortion rates (5.14% vs. 8.08%, $P = 0.268$) were also found to be lower in the IVF group, although the difference was statistically insignificant [Table 2].

The total fertilization failure rate in the IVF group was 0.5% (1/214) in IVF and 0% (0/136) in the ICSI group.

Discussion

The introduction of ICSI in male factor infertility was a major breakthrough in the field of assisted reproductive techniques (ART). It increased the possibility of conception in couples with severe male factor infertility.

Table 1: Comparison of demographic statistics between two groups (*n*=350)

Baseline characteristics	Procedure		*P*
	IVF (*n*=214)	ICSI (*n*=136)	
Wife age	30.71±3.45	31.96±4.1	0.460
Estradiol value on the day of hCG E2 (pg/ml)	2162.66±796.47	2094.98±748.65	0.428
Progesterone value on the day of hCG (ng/ml)	0.98±0.38	0.9±0.37	0.055
Endometrium thickness (mm)	10.28±1.85	10.15±1.74	0.504
Number of oocytes retrieved	12.45±5.54	11.08±5.49	0.128
Number of MII oocytes	10.78±4.9	7.82±4.15	<0.001
Number of good quality embryos for transfer or freezing	4.42±2.98	3.15±2.11	<0.001
Number of embryos transferred	1.72±0.55	1.72±0.56	0.844
Fertilization rate (%)	89.99	85.1	<0.001
Cleavage rate (%)	94.41	92.7	0.0001
Blastocyst formation rate (%)	62.86	50.61	<0.001

MII=Metaphase II, hCG=Human chorionic gonadotropin, E2=Estradiol, IVF=*In vitro* fertilization, ICSI=Intracytoplasmic sperm injection

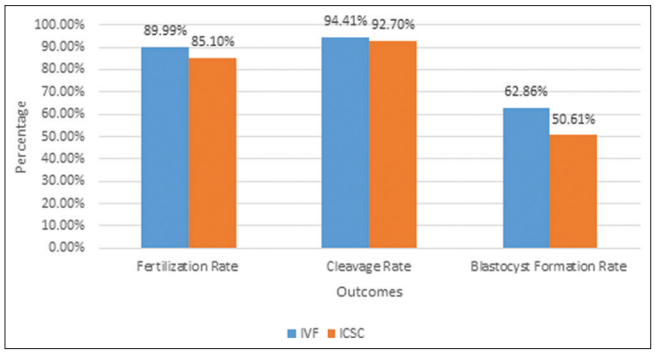

Figure 1: Cluster bar graph for comparison of various outcome parameters

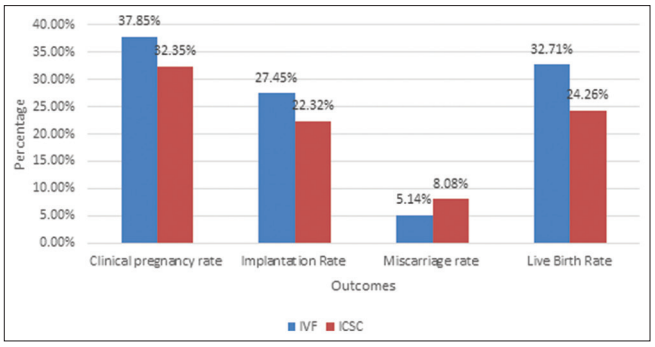

Figure 2: Cluster bar graph for comparison of various outcome parameters between two groups

Table 2: Comparison of various outcome parameters between two groups (*n*=350)

Parameter	Procedure		*P*
	IVF (*n*=214)	ICSI (*n*=136)	
CPR (%)	37.85 (81/214)	32.35 (44/136)	0.295
IR (%)	27.45 (101/368)	22.32 (52/233)	0.159
Miscarriage rate (%)	5.14 (11/214)	8.08 (11/136)	0.268
LBR (%)	32.71 (70/214)	24.26 (33/136)	0.09

IVF=*In vitro* fertilization, ICSI=Intracytoplasmic sperm injection, LBR=Live birth rate, IR=Implantation rate, CPR=Clinical pregnancy rate

However, a vast majority of ART clinics are currently doing ICSI as a routine procedure irrespective of male factor. The primary reason is probably to circumvent the occasional problem of facing total fertilization failure (TFF) in conventional IVF.

In our study, we compared the reproductive outcomes of IVF against ICSI in cases where male factor was normal. It was observed that although similar numbers of oocytes were retrieved in both the groups, the IVF group had significantly higher number of mature oocytes. One possible explanation for this difference in the number of mature oocytes could be that in ICSI cycle, oocytes were checked for maturity on insemination, and immature oocytes were discarded but in standard IVF, the maturity of oocytes was not examined until 16–18 h after insemination which gave scope for some of the immature oocytes to mature in culture. The cumulus–oocyte complex was maintained intact in culture, allowing more oocytes to complete maturation and subsequently achieve fertilization. Our study found that fertilization rate per oocyte retrieved and blastocyst formation rate were higher in IVF group, which was statistically significant. This indicates that IVF allows the natural selection of most robust sperm with maximum fertilization capacity to penetrate the oocyte.

In an RCT by Bhattacharya *et al*., 415 couples with nonmale factor infertility were randomized to IVF and ICSI. It was found that the IVF group had a higher number of inseminated oocytes and a higher fertilization rate per collected oocyte, despite a similar number of oocytes retrieved (11 oocytes in each group).[12] Other studies have also reported similar findings.[13-16] The inferior results with respect to fertilization and zygote formation rate in ICSI could be explained by the oocyte degeneration, which might result from mechanical damage occurring during the ICSI procedure. In various studies, it was observed that this damage can occur in 5%–19% of injected oocytes.[17-19] The competence of the embryologist could be another factor affecting the success rates of ICSI. Inaccurate placement of injection pipette can lead to meiotic spindle damage causing a detrimental effect on fertilization.

In our study, we found that total fertilization failure was 0.5% in the IVF group and 0% in the ICSI group for the nonmale factors cases. Taylor *et al*. too found no difference between the incidence of TFF in IVF (4%) and ICSI (4.5%).[20] TFF in IVF is mostly due to sperm abnormalities and TFF in ICSI could be due to some inherent, subtle abnormalities in the form of oocyte factors such as thick zona pellucida and oocyte activation failure. ICSI carries a lesser risk of TFF, and the reported incidence is 2%–3%. This shows that to prevent one case of TFF in IVF; we need to do 33 extra cases of ICSI, thereby putting a big question mark on the rationale of using ICSI as a routine in couples with normal malefactor.[12]

The results in IVF group edged over ICSI group (although not statistically significant) with respect to fertilization rate, blastocyst formation rate, IR, CPR, and LBR.

Our results were similar to those of previous studies in terms of pregnancy and fertilization rates.[12,16,21-23] Recently, in a new cohort study conducted in Australia, Li *et al*. studied the use of ICSI in nonmale factor treatments and again found no benefit in LBR for ICSI. A cohort of 14,693 women having IVF and ICSI between 2009 and 2014 in the state of Victoria, Australia, were studied, and the results were based on the outcome of

treatment. Clinical pregnancies and live births were recorded for the first oocyte retrieval (fresh stimulated cycle and associated thaw cycles) until a live birth was achieved, or until all embryos from the first oocyte retrieval had been used. Similar to the trend around the world, ICSI usage was more (8470 women) than the use of IVF (4993 women). Over the study period, the use of ICSI increased from 52.6% in 2009 to 65.9% in 2014, whereas the proportion of couples with male factor infertility remained relatively stable. The cumulative outcome in each group was no better or worse than the other (37% LBR for IVF and 36% for ICSI).[24]

The European IVF International Monitoring report of 2013 reported a CPR of 34.5% per ET with IVF and 32.9% per ET with ICSI. These rates were described as "stable." Despite all these evidence, ICSI is still the world's favored method of fertilization irrespective of indication, with around two ICSI cycles performed for every one of IVF.[25,26]

Moreover, there are studies which have shown that the perinatal outcomes with ICSI are suboptimal in the form of increased birth defects, congenital anomalies, and epigenetic changes.[27-29]

We also found that the expenditure incurred for ICSI cycle was 5% higher due to laboratory consumables compared with conventional IVF cycle. This was similar to world statistics which quote the same figures of around 5%–8%.[30] The laboratory time and working hours for the embryologist in ICSI cycle is directly proportional to the number of oocytes retrieved per cycle, whereas in conventional IVF the time and working hours remain fairly the same.

The routine use of ICSI over IVF has become a standard practice in the modern ART. There are no guidelines to follow a single method in nonmale factor infertility. In 2012, the ASRM also declared that "there is no data to support the routine use of ICSI for nonmale factor infertility."[6] Similarly, one of the key editors of ESHRE said that the effect of ICSI is clearly overestimated and that its use in the majority of nonmale factor infertility was unnecessary, ineffective, and expensive.[31]

The strength of our study was the follow-up of pregnancies till live birth, which is the ultimate goal of any ART procedure. The major limitation of our study was the limited sample size, lack of randomization due to its retrospective nature, and lack of data on birth defects and neonatal outcomes.

Conclusion

Currently, there is no evidence demonstrating any added advantage of the routine use of ICSI over IVF in couples with normal malefactor. However, more prospective well-designed randomized control trials would be needed in future to formulate strong evidence-based guideline regarding the position of ICSI in nonmale factor infertility. As of today, the routine use of ICSI in infertile couples with normal male factor is certainly not justified.

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Conflicts of interest

There are no conflicts of interest.

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