Original Article

Relevance of Split *In vitro* Fertilization-Intracytoplasmic Sperm Injection Method of Insemination in Normozoospermic and Mildly Oligospermic Men: A Retrospective Study

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Abstract

Background: Use of intracytoplasmic sperm injection (ICSI) is generally considered redundant in cases of normozoospermia, or mild male factor cases of infertility and conventional method of insemination is advocated. However, there is a risk of low fertilization or total fertilization failure (TFF) and to avoid this, split *in vitro* fertilization (IVF)-ICSI method of insemination is advised. In our study, we have shown that not only TFF is avoided with split method of insemination, but also cancellation of embryo transfer (ET) can be avoided in a significant number of IVF cycles. **Aims:** This study aimed to assess whether the IVF-ICSI split insemination method was able to reduce the risk of ET cancellation in couples with normal or mild sperm characteristics. **Settings and Design:** It is a retrospective study including a total of 107 split insemination cycles done at our center. **Materials and Methods:** The female partner's age was under 37 years, and at least ten oocytes were retrieved in all cycles. Sibling oocytes were randomly allocated to IVF or ICSI. Statistical analysis was carried out using Graphpad Prism, Instat. **Results and Conclusion:** The fertilization rate in oocytes kept in conventional IVF was significantly higher (79.8%) compared to that of oocytes injected through ICSI (69.1%). Only one couple had TFF. In majority of the cycles, i.e., 97 out of 107 cycles, the mode of insemination did not affect the fertilization rate or embryo quality. Nearly 28% of the cycles were saved from ET cancellation by adopting the split insemination method. "Split IVF-ICSI" approach can save a significant number of ART cycles and is found to be cost-effective as it avoids incurring the cost of two ART cycles.

Keywords: *Conventional fertilization, intracytoplasmic sperm injection, split insemination, total fertilization failure*

Introduction

In an *in vitro* fertilization (IVF) cycle, insemination can be done either by conventional IVF or by intracytoplasmic sperm injection (ICSI). Conventional IVF has been the method of choice in cases of normozoospermia. Sometimes, there is a risk of total fertilization failure (TFF) with this method and in such cases, ICSI has helped in achieving improved fertilization rates.[1]

From an embryologist's perspective, an IVF cycle is considered complete if we have good-quality embryos for transfer on day 3 or day 5. The whole laboratory exercise is a waste if we end up with poor quality embryos which are not transferable or if no embryos are available due to total fertilization failure. It is

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devastating for the patient as well as for the clinician and the embryologist involved. More so, despite the progress made in the field of IVF, it is still very difficult to predict fertilization failure or poor fertilization in conventional IVF cycles even with normal semen parameters. To avoid such a situation, one can opt for split IVF-ICSI insemination in sibling oocytes, provided the male factor comes in the category of "normozoospermia." Such cases generally have unknown etiology and have unexplained infertility. Unexplained infertility factors can be a hidden male factor despite normal semen parameters, an oocyte factor, and uterine factors such as endometrial receptivity.

ICSI was introduced to tackle cases with severe male factor infertility where conventional IVF was not possible.[2,3] However, there is redundant use of ICSI across the world in cases where it is not even required. In addition, the role of ICSI in mildly oligospermic and in normozoospermic men having low or no fertilization rates using the conventional method of fertilization is not strongly established yet.[4] One of the major concerns in treating couples with IVF treatment is complete fertilization failure, which is reported to occur in 4%–50% of the couples and thus, it is tempting to propose ICSI to such couples as a means of decreasing the risk of failed fertilization.[2,5,6] ICSI, however, has unresolved concerns regarding the long-term outcomes of the IVF conceptions in terms of epigenetic consequences.[7]

Several studies have shown that conventional IVF and ICSI performed on sibling oocytes (the IVF-ICSI split procedure) in patients with unexplained infertility[8] or in cases with previous unexplained fertilization failure[9,10] reduces the risk of complete fertilization failure. The oocyte maturity, dysmorphisms, and oocyte quality to a certain extent are definitely better assessed using ICSI, and these data may be useful to assess the adequacy of the stimulation protocol used. Furthermore, it has been shown that implementation of ICSI in couples with mild male factor infertility could improve fertilization rates and decrease the risk of complete fertilization failure.[11] This study also implicated that split ICSI procedure provides valuable clinical information about fertilization potential for the couple so that unnecessary use of ICSI can be avoided in future cycles of patients achieving good fertilization rates in both IVF and ICSI.

The purpose of this retrospective study was to assess whether the performance of the IVF-ICSI split insemination method was able to reduce the risk of ET cancellation in couples with normal sperm characteristics with improved fertilization rates and embryo quality apart from reducing the risk of TFF.

Materials and Methods

This retrospective study included all IVF cycles at our center from October 2016 to March 2018, in which sibling oocytes were randomly divided for ICSI or IVF insemination. This method is a routine practice in our unit in cases of mildly oligospermic or normozoospermic cases if we retrieved ≥12 oocyte cumulus complexes (OCCs) or ≥10 metaphase II (MII) oocytes with previous at least two intrauterine insemination failures.

Controlled ovarian stimulation using recombinant follicle-stimulating hormone (Gonal-F; Merck, Serono) with gonadotrophin-releasing hormone antagonist protocol (cetrorelix) and human chorionic gonadotrophin (HCG) was carried out in case of all the patients. Cycles were monitored with follicular ultrasound measurements and serum estradiol concentrations from day 6 of stimulation start. HCG trigger was given when two or more follicles had a diameter of ≥21 mm. Egg retrieval was conducted by transvaginal ultrasound 36 h after HCG administration.

OCCs were picked from the follicular fluid and pipetted into a Falcon dish (BD 3001)) with 2 ml of HEPES-buffered G-MOPS™ Plus medium (Vitrolife, Sweden) covered with 2 ml of OVOIL™ (Vitrolife). Once ovum retrieval was completed, all the OCCs picked were washed once again in G-MOPS™ Plus medium in a center well dish (BD1007). A second wash was given in G-IVF™ Plus medium in a center well dish. After that, the OCCs were transferred to G-IVF™ Plus medium in center well for further incubation of 2–3 h. At this stage, the OCCs were allocated to IVF or ICSI groups without any bias except that for IVF, selection was made among good-looking OCCs.

Semen samples were prepared using double-density gradient (40% and 80%) followed by a single wash by Sperm Wash™ medium (Sage). Then, swimup was given in the same spermwash medium for 30–45 min to collect 100% motile sperms. The sample thus prepared was used for both IVF and ICSI.

Denudation dish was prepared in 4-well Nunc™ dish. The first well contained 0.6 ml of hyaluronidase 80 IU/mL (Sage) covered with OVOIL™. The rest of the three wells were filled by 0.6 mL of G-MOPS™ Plus covered with Ovoil. After 2–3 h incubation after egg retrieval, OCCs allocated for ICSI were denuded by a short exposure to HEPES-buffered medium with 80 IU/ml hyaluronidase for 1 min. Then, the oocytes were stripped with a 170-µm Denupet in G-MOPS™ plus medium in the 2nd well. After this, the oocytes were transferred to the 3rd well and were further denuded using 130-µm Denupet and after

giving final wash in G-MOPS™ Plus in the fourth well, the oocytes were transferred to the postdenudation dish containing G-1 Plus™ medium covered with OVOIL™ in a center well and placed back into the incubator (37°C) for 30 min to 1 h until ICSI.

For conventional IVF, not more than eight OCCs were kept in a central well in G-IVF™ Plus covered with Ovoil. For insemination of the oocytes, 50,000–60,000 sperms per oocyte were added.

For ICSI, MII oocytes were injected 2–3 h post egg retrieval, with the first polar body either at the 12 o'clock or 6 o'clock position.[12] The sperm injection was carried out at 3 o'clock position with some aspiration of the cytoplasm with a hint of oolemma breakage and then finally, the sperm and cytoplasm were expelled back into the oocyte.

Fertilization check and oocyte survival was carried out at 17–19 h post insemination, ICSI, or conventional IVF. Fertilization was considered normal when two pronuclei were seen in combination with two individualized or fragmented polar bodies. The degenerated or unfertilized or abnormally fertilized oocytes were separated from the normally fertilized oocytes. Embryos' image capture and grading was done at 42–46 h for day 2 and 66–70 h for day 3 post insemination. The grading involved the blastomere symmetry, day-specific number of blastomeres, and percentage of fragmentation. Accordingly, day-3 embryos were given a grading of A, B, or C, where A was the best-quality embryo and C was the poor-quality embryo, which were generally discarded. Grade A embryos on day 3 had at least seven cells with blastomere symmetry and <10% fragmentation, Grade B embryos showed 10%–30% fragmentation, and Grade C had asymmetry of the blastomeres along with >30% fragmentation. On day 3, A- and B-grade embryos were either transferred or frozen for the patient.

Statistical analysis was carried out using Graphpad Prism, Instat software (GraphPad Software Inc, San Diego, USA).

Results

A total of 1764 OCCs were retrieved from 107 IVF cycles. A total of 1037 OCCs were denuded, of which 996 were found to be mature and were subjected to ICSI. A total of 727 OCCs were subjected to IVF. Fertilization rate, cleavage rate, and Grade-A embryos were compared in these split IVF-ICSI cycles [Table 1]. The fertilization rate in oocytes kept in conventional IVF was significantly higher (79.8%) compared to oocytes injected through ICSI (69.1%). Although the fertilization rate is expected to be higher in ICSI, we got a lower rate, which can be attributed to poor oocyte quality and thus high degeneration of oocytes post-ICSI in a few cases.

We had one couple with TFF, in which none fertilized either in IVF or ICSI. The male factor was excluded as the sperms were able to fertilize the donor oocytes. Therefore, an occult oocyte factor was considered to be the player in this case of TFF.

Both the cleavage rate and embryo quality were better for the conventional IVF group of oocytes and were found to be statistically significant [Table 1]. Hence, in majority of the cycles, i.e., 97 out of 107 cycles, the mode of insemination did not affect fertilization rate or embryo quality.

There were 11 cycles where ICSI was a better performer as in these cycles, the IVF group of oocytes either showed no or low fertilization or had poor-quality embryos. The comparison is shown in Table 2.

There were 19 cycles where the IVF counterpart performed better than the ICSI inseminated sibling oocytes [Table 3].

Table 1: Comparison of different parameters between *in vitro* fertilization and intracytoplasmic sperm injection in sibling oocytes

Parameter	IVF	ICSI	*P*
Fertilization rate	580 (79.8)	688 (69.1)	0.0007
Cleavage rate	522 (90.1)	614 (89.2)	1.0000
Grade A embryos*	318 (60.9)	277 (45.1)	0.0002

*Day-3 embryos which had at least seven cells and <10% fragmentation. Values given in parentheses are percentages. IVF=*In vitro* fertilization, ICSUI=Intracytoplasmic sperm injection

Table 2: Cases where intracytoplasmic sperm injection performed better than *in vitro* fertilization (total no. 11/107)

	ICSI	IVF	*P*
Fertilization rate	74 (76)	21 (32)	<0.0001
Embryo quality	39 (53)	14 (33)	0.0169

The total number of MII oocytes in ICSI was 98 and in IVF was 66 in these 11 cycles. Values given in parentheses are percentages. MII=Metaphase II, IVF=*In vitro* fertilization, ICSI=Intracytoplasmic sperm injection

Table 3: Cases where *in vitro* fertilization performed better than intracytoplasmic sperm injection (total no. 19/107)

	ICSI	IVF	*P*
Fertilization rate	91 (54)	99 (89)	<0.0001
Embryo quality	40 (44)	81 (82)	<0.0001

The total number of MII oocytes in ICSI was 168 and in IVF was 111 in these 19 cycles. Values given in parentheses are percentages. MII=Metaphase II, IVF=*In vitro* fertilization, ICSI=Intracytoplasmic sperm injection

The embryos in the current study were transferred based on embryo quality regardless of their origin from ICSI or conventional IVF. The overall clinical pregnancy rate in these split IVF-ICSI cycles was 63.5%. Live birth rate and miscarriage rates were 42.6% and 7.3%, respectively.

Discussion

In a country like India, where assisted reproductive technology (ART) procedures are still not covered by government funding schemes and with patients' high expectations from these procedures with remarkable financial and emotional involvement, there is tremendous pressure on the whole ART team to at least complete the cycle with good embryo transfer (ET). ICSI is generally performed in the subsequent cycle of once-failed conventional IVF. In addition, it is difficult to choose between ICSI and conventional IVF as a method of insemination for the retrieved oocytes. Hence, to provide them with the best possible in the same cycle, we do split ICSI-IVF inseminations to avoid a situation of TFF or oocyte degeneration post-ICSI due to poor oocyte quality. As the probability of poor or no fertilization is high in conventional IVF, lesser number of oocytes were kept in the conventional IVF group compared to ICSI group.

Precise comparison of the clinical outcomes of IVF versus ICSI cycles is not possible as each procedure deals with different infertility symptoms. There are studies which have compared IVF versus ICSI outcomes as far as fertilization rate and embryo quality are concerned. A study by Hsu *et al*. showed that IVF-derived day-3 embryos scored better than ICSI-derived day-3 embryos.[13] It has also been demonstrated that embryos obtained through ICSI had decreased development potential when cultured till blastocysts.[14] Studies have shown that embryo quality does not seem to be affected by the mode of fertilization (IVF or ICSI).[15-17] Ou *et al*. showed that embryo quality does not get affected by the fertilization process *per se,* but depends on intrinsic factors of the gametes involved.[18] All the above-mentioned studies did not compare IVF- or ICSI-derived embryos in the same cohort of oocytes or sibling oocytes.

A study by Tobler *et al*. demonstrated that in the sibling oocytes, ICSI did not confer any additional advantage over conventional IVF as far as fertilization, cleavage, blastulation, and pregnancy rates are concerned in couples with normozoospermic semen undergoing their first IVF cycle. Their study also showed an overall trend for improvement in the conventional IVF group over the ICSI group in the parameters studied though not statistically significant.[19]

Li *et al*. investigated whether half-ICSI is of any use to patients with unexplained infertility and found that ICSI of all the oocytes would be better option for such patients.[20] Another study found performance of ICSI to be better compared to that of conventional ICSI in sibling oocytes as far as the fertilization rate and embryo quality rates were concerned.[21]

In our study, we found that in some patients, the embryo quality was not affected by the method of insemination. The reason behind this is not clear except that in cases where oocyte quality is very poor; an invasive process such as ICSI may lead to poor-quality embryo. In addition, in some cases, ICSI-derived embryos were better than IVF-derived embryos. The reason can be better sperm selection and thus better embryo development.

Our results show that fertilization rate, cleavage rate, and embryo quality were all better in IVF-inseminated oocytes as compared to ICSI [Table 1]. The first study in sibling oocytes by Shveiky *et al*., found that fertilization rates by ICSI were lower in the essential ICSI compared with the nonessential ICSI group, at 65% and 73% ($P < 0.025$), respectively, in sibling oocytes.[22] This observation was similar to our study (79.8% in IVF and 69.1% in ICSI; $P < 0.005$). ICSI is shown to increase fertilization rates.[1] The better performance of IVF in our study can be due to biased preselecting good-looking OCCs for conventional IVF. The lower fertilization rate in ICSI obtained in our study can also be attributed to higher degeneration of oocytes post-ICSI due to poor oocyte quality not fertilization failure *per se.*

The data analysis shows that we would have completely lost the chance of ET in almost 10% of the patients if we had done conventional insemination in all the oocytes using IVF [Table 2]. In these patients, either we had poor fertilization or poor embryo quality. Similarly, 18% of the patients would not have undergone ET in case all ICSI had followed [Table 3]. Eight percent of these cycles were those where there was no embryo formed worth transfer after ICSI. In total, 28% of the patient cycles got benefitted by the use of "split IVF-ICSI" protocol.

Conclusion

Therefore, "split IVF-ICSI" approach has saved a significant number of ART cycles and is a promising approach to follow in normozoospermia and mildly oligozoospermia cases. This approach is preferred from the cost-effectiveness point of view also, as it avoids incurring the cost of two ART cycles. i.e., one failed IVF cycle and then subsequent ICSI cycle or *vice versa.*

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Conflicts of interest

There are no conflicts of interest.

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