ICSI AND CONVENTIONAL IVF, WHICH ONE SHOULD BE PREFERED FOR CASES OF PRE-IMPLANTATION GENETIC TESTING?

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Ever since its implementation in 1992, the intra-cytoplasmic sperm injection (ICSI) technique has been hailed as a game-changer for enhancing the outcomes of in vitro fertilization (IVF) treatment in cases of infertility due to male factors. However, ICSI was later widely applied to cases of infertility not due to male factors without evidence proving its effectiveness. According to the 2022 report of the European IVF Monitoring Council (EIM), the rate of fertilization cases using ICSI method accounts for 70% of IVF cases in European countries, meanwhile the conventional IVF method accounts for only 30% and this rate has remained stable from 2006 until recently. The question is whether cases of infertility not due to male factors that are indicated for pre-implantation genetic testing (PGT) should be fertilized and create embryos using ICSI or conventional IVF to minimize invasiveness and reduce costs while still ensuring the accuracy of the test results.

Keywords: Intra-cytoplasmic sperm injection (ICSI), conventional in vitro fertilization (conventional IVF, conventional IVF), pre-implantation genetic testing (PGT), trophectoderm biopsy, genetic contamination.

I. INTRODUCTION

Infertility has a worldwide impact, affecting around 48.5 million pairs of individuals, which adds up to 186 million people.[1,2] As a result, assisted reproduction technology (ART) has become the preferred method for those seeking reproductive assistance. In the span of close to 40 years, impressive headway has been accomplished, with the International Committee for Monitoring ART (ICMART) revealing that the total number of infants conceived through ART techniques and modern fertility therapies worldwide has surpassed 8 millions, more than four decades since the advent of the first IVF baby.[3] Furthermore, progress in stimulating ovaries, retrieving eggs, cultivating and evaluating embryos, as well as in methods for freezing, have been key factors in significant advancements in treatments for human fertility. The development of intracytoplasmic sperm injection stands out as a significant technological breakthrough in the field of ART, aimed at addressing the issue of low and inconsistent fertilization rates encountered in conventional IVF when faced with poor sperm quality.[4] Still, the last twenty years have shown a notable increase in the frequency of ICSI procedures. Many countries worldwide have seen a significant uptick in this trend, especially in the Middle East where the ICSI utilization rate is nearing 100%. From 1996 to 2012, there was a notable increase in the application of ICSI in the United States, climbing from 36% to 76%.[5]

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Received: 12/09/2024
Accepted: 01/10/2024

The method of fertilization sets conventional IVF apart from ICSI: in conventional IVF, eggs are exposed to sperm in a dish for fertilization, whereas in ICSI, a chosen sperm is injected into the egg by an embryologist. So it can be easily distinguished between an embryo created by conventional IVF and an embryo created by ICSI. Despite the widening of the initial conditions for ICSI, there has been no corresponding enhancement in the outcomes of ART.[6] According to the guidelines of the National Institute for Health & Care Excellence (NICE), ICSI should be considered when there are significant issues with sperm quality or if previous IVF attempts have been unsuccessful or resulted in very low fertilization rates.[7] The Practice Committees of the American Society for Reproductive Medicine (ASRM) suggested that ICSI for non-male-factor indications is a process that demands more time and resources when contrasted with conventional IVF.[8] The safety of ICSI is being questioned because it seems to be connected to a greater likelihood of congenital abnormalities and autism when contrasted with conventional IVF, despite the unclear biological mechanisms involved.[9] In general, there is a discrepancy between the widespread application of ICSI in couples without male infertility and the supporting evidence. With the additional financial burden and invasive features of this method in mind, it is necessary to meticulously evaluate the implementation of ICSI in ART, while professionals in the field should critically analyze conventional IVF.

The identification of genetic abnormalities in embryos before implantation is made possible through preimplantation genetic testing (PGT), an advanced tool in assisted reproduction. The purpose of PGT is to analyze embryos for chromosomal abnormalities, monogenic diseases, and structural rearrangements. Since its introduction by Handyside et al in the United Kingdom in 1990 for sexing embryos, PGT has been conducted worldwide. Before 2010, the common practice was to perform cleavage stage embryo biopsy along with fluorescent in situ hybridization (FISH), but by 2012, the focus shifted to blastocyst biopsy specifically on the trophectoderm. In addition, array comparative genomic hybridization (aCGH) was utilized for analysis and later upgraded to next-generation sequencing (NGS), which is employed worldwide. PGT includes PGT-A, PGT-M, and PGT-SR as its primary forms. PGT-A is employed to examine embryos for aneuploidies, PGT-M is employed for identifying single gene disorders, and PGT-SR is used for identifying chromosomal abnormalities due to structural rearrangements in the genome. The main focus of PGT-A has been on IVF patients, aiming to boost pregnancy rates per embryo transfer and lower the incidence of miscarriages. Recently, there have been new measures implemented, such as promoting elective single embryo transfer and reducing the time taken to conceive. Advanced maternal age, recurrent implantation failure, severe male factor, and recurrent miscarriage in couples with normal karyotypes are cited as reasons for considering PGT-A.[10]

The quality of the biopsy sample plays a significant role in determining PGT-A test results. And there are various factors such as the quality of the embryo, the process of embryo biopsy, the tubing and transportation of samples, and the genetic testing procedure… can affect that quality. In which, it is necessary to consider the possibility of potential contamination of genetic material during embryo biopsy which is specific for embryos created through conventional IVF insemination.

So the question is whether ICSI should be

performed for non-male factor infertility cases with PGT-A indication and what evidence supports this? The objective of this analysis was to review current evidence about the possibility of male genetic material contamination in cases of non-male factor infertility patients who were indicated for PGT-A. In these cases, we should fertilize and create embryos using ICSI or conventional IVF to increase efficiency, minimize invasiveness and reduce costs while still ensuring the accuracy of the test results.

II. OVERVIEWS

1. Current evidence

Risk of genetic material contamination

According to ESHRE's 2020 recommendations and for embryo biopsy for PGT, ICSI is the recommended technique for PGT to lower the risk of contamination from cumulus cells and surplus sperm on the zona pellucida.[10] It is crucial to delicately remove cumulus cells and wash oocytes before ICSI, and rinse zygotes post-fertilization in IVF to prevent any leftover maternal material in the biopsy samples. The consensus on ICSI in non-male factor infertility issued by the ASRM in 2020 also mentioned the application of the ICSI method in cases where PGT is indicated and there is a risk of genetic material infection transmitted from sperm.[8] The reason for employing ICSI was to guarantee fertilization by a single sperm and prevent the risk of contamination from additional sperm on the zona pellucida when utilizing polymerase chain reaction.[11] This is now a minor issue due to the advancements in molecular techniques like next-generation sequencing. The report, as expected, showed that there were no variance in the division and excellence of embryos generated from regular zygotes with the use of any of the fertilization techniques. In a separate analysis, there was no substantial difference in aneuploidy rates or mosaicism noted when contrasting fertilization methods, although the literature is deficient in relevant details.[12] Thus, in cases where male infertility is not a concern, ICSI for PGT should be limited to situations where the test results could be affected by the introduction of unrelated sperm.

However, some recent studies show that conventional IVF method can be applied to cases of infertility not due to male factors for which PGT-A testing is indicated. Lynch's study showed that there was no evidence of sperm DNA being amplified during Whole Genome Amplification (WGA) process when sperm was present in the analyzed sample.[13] The research involved collecting semen samples from five men with normal sperm parameters. The process involved gathering 1, 2, 4, 8, and 10 sperm from each sample into PCR tubes, followed by performing WGA according to PGT-A processing guidelines. The findings indicated that there was no DNA amplification detected in any of the 25 samples, which contained a combined amount of 125 sperm. The author believes that the reason for this is the distinct method in which sperm DNA is structured, rendering it unreachable in the entire genome amplification process unless proper measures are taken to decondense and separate the DNA. In details, haploid sperm DNA undergoes compaction and inactivation within the nucleus as part of spermiogenesis. Transition proteins and protamines play a role in condensing chromatin, and seminal plasma enhances chromatin stability post-ejaculation. The nucleus of the oocyte remains in a extremely compact and not functioning state following the penetration of a sperm. Inside the ooplasm, there is a substance that helps sperm DNA to unpack, and it is only when the sperm

and oocyte membranes fuse that the sperm DNA becomes available, as certain proteins in the ooplasm replace others, allowing for DNA unpacking, formation of a pronucleus, DNA duplication, and the start of mitotic phase.[14] Thus, an intense approach to cell lysis is required before WGA when endeavoring to amplify single sperm through library-based techniques. The usual procedure includes using proteinases and DTT at increased temperatures to induce chromatin decondensation.[15-17] Consequently, it is not unexpected that sperm did not undergo amplification with the standard NGS method. Thus, the possibility of genetic contamination in the conventional IVF method resulting in adverse events or misdiagnoses in PGT-A is slight.

De Munck et al also performed an experiment on WGA of sperm cell.[18] The collection of sperm cells was done in a washing solution and then placed in tubes under the same conditions as the TE samples (Figure 1).

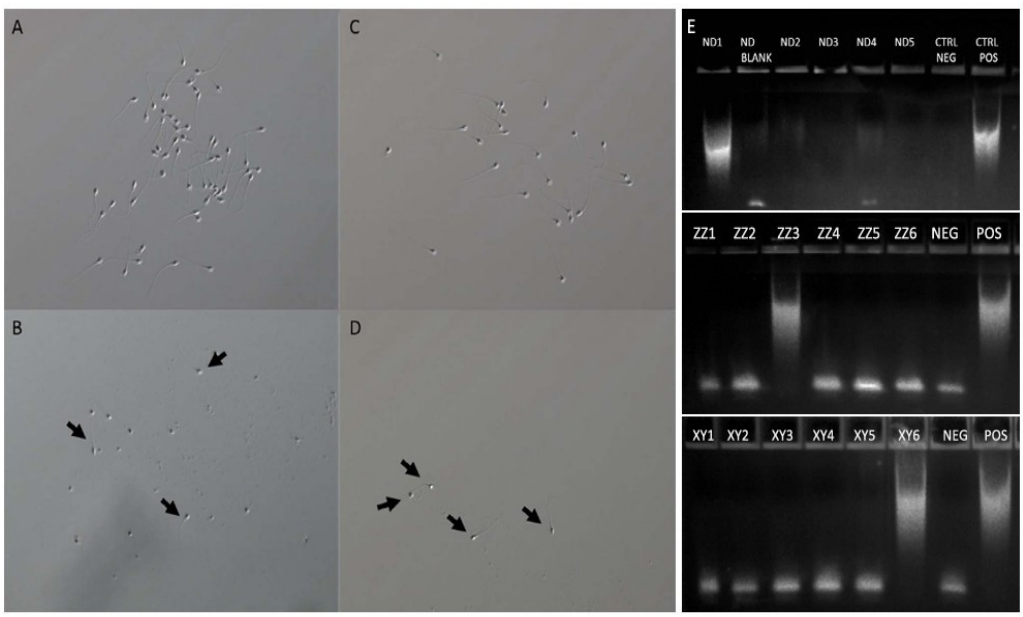

Figure 1. Validation whole genome amplification of sperm and results[18]

It was found in the experiment that the WGA protocol was ineffective in amplifying sperm DNA, even with 60 sperm cells included. The outcomes were consistent with what Lynch discovered in their previous investigation.[13]

On the other hand, during the embryo culture process after fertilization until the blastocyst stage, the remaining granulosa cells on the zona pellucida are also largely eliminated. During the process of performing embryo biopsy and washing blastocyst samples, the embryologist always avoids granulosa cells as much as possible, so the risk of contamination with genetic material from granulosa cells is also minimized.

Consequently, considering the most recent scientific data, the likelihood of genetic material tainting from either the father or mother is exceedingly slight.

PGT-A results after ICSI and conventional IVF

Comparing the rate of euploid embryos after PGT-A between the group of embryos fertilized with ICSI and the group of embryos

fertilized with conventional IVF, some authors concluded that the rate of euploid embryos after PGT-A is no different between the group of patients fertilized by ICSI and the group of patients fertilized by conventional IVF.[12,18,19] Palmerola and colleagues conducted a study in 2018 comparing rates of euploid, aneuploid, and mosaic embryos in trophectoderm biopsy samples from IVF and ICSI PGT-A cycles.[12] A total of 302 PGT-A cycles were analyzed, with 75 coming from IVF and 227 from ICSI procedures, leading to the biopsy of 251 IVF and 724 ICSI blastocysts. The results of PGT-A were similar in IVF and ICSI cycles, with euploid rates of 27.9% for IVF and 30% for ICSI, aneuploid rates of 45.4% for IVF and 43.1% for ICSI, and inconclusive results of 4.4% for IVF and 6.2% for ICSI. A slight difference was noted in the rate of mosaicism between IVF (25.9%) and ICSI (20.9%), although it was not considered significant.

In De Muck's study, a total of 568 COCs from 30 infertile couples were randomly allocated between IVF (n = 283) and ICSI (n = 285).[18] There was no difference in the blastulation rate on day 5 between the two insemination methods for embryos cultivated until they reached the blastocyst stage (8.4 vs 70.8%; p = 0.076; OR: 1.10062 [0.99364 - 1.21919]). No discrepancy was found in the average euploid rate per cycle when comparing conventional IVF with ICSI (49.8 vs 44.1%, p = 0.775; OR: 1.05664 [0.75188 - 1.48494]).

In Jie Deng's investigation, a total of 2,129 oocytes were split between conventional IVF (1,026) and ICSI (1,103).[19] Aneuploidy rates and percentages of mosaic embryos per biopsy were similar between conventional IVF and ICSI sibling oocytes, with no significant difference noted (50.3% vs 45.2% and 1.7% vs 2.4%, respectively). The percentages of various types of aneuploidy and aneuploidies with sex chromosome abnormalities were comparable in both groups, at 6.2% and 7.2%, respectively. The final outcome showed that the likelihood of having aneuploidy embryos per assigned oocyte was comparable in both groups, at 13.2% versus 15.5%.

Most recently there is Patel's study published in 2023 with the largest number of participating patients.[20] The research aimed to determine if there are differences in euploidy rates between ICSI and conventional IVF for cases of infertility not related to male factors. It was concluded by researchers that in cases of infertility that were not due to male factors, ICSI led to a reduced fertilization rate and an 11% decrease in embryo euploidy compared to traditional IVF. Despite adjustments made for the PGT reference laboratory, the findings still supported the idea that ICSI did not confer any benefits.

So there is no added benefit of ICSI compared to conventional IVF when it comes to the rate of euploid embryos following PGT-A testing in infertility cases not involving male factor.

Live-birth rates after ICSI and conventional IVF

In the 2020 ASRM committee opinion regarding ICSI for indications other than male infertility, it was noted that the growing utilization of ICSI did not result in better live-birth rates.[8] In 2018, a study based on a population group supported this idea by showing a comparable overall success rate in live births when comparing ICSI to conventional IVF for couples without male infertility issues.[21] Song et al conducted a retrospective cohort study on 549 IVF and 241 ICSI cycles for individuals with unexplained infertility at a university hospital's fertility center between 2016 and 2018.[22] The

study aimed to analyze the differences in live-birth and clinical pregnancy rates between the two groups. The live-birth rate in the IVF group was determined to be 35.2% (172 out of 488), while in the ICSI group it was 33.3% (65 out of 195), with a p-value of 0.635. The clinical pregnancy rates, implantation rates, and miscarriage rates were comparable between the two groups. Moreover, ICSI did not result in higher live-birth rates when treating unexplained infertility, but it did cause more cancellations than standard IVF.

A randomized controlled trial by Vinh et al in 2021 aimed to determine if intracytoplasmic sperm injection led to a higher live-birth rate than conventional IVF.[23] 1064 couples were randomly assigned to ICSI (n=532) or conventional IVF (n=532). The occurrence of live birth following the first embryo transfer from the initiated cycle was 35% for couples randomly assigned to intracytoplasmic sperm injection and 31% for those assigned to conventional IVF, with a small absolute difference of 3·4%. Overall, there was no significant improvement in the chances of live birth or other pregnancy results when comparing ICSI to traditional IVF in cases of infertility where the male partner has normal sperm count and motility. It is necessary to reconsider the regular application of ICSI in assisted reproduction for this group because of the extra expenses and intrusive procedure involved.

A retrospective cohort study carried out by Iwamoto et al in 2022 examined the influence of the ICSI technique on cumulative live-birth rates in a significant sample.[24] The research compared the cumulative live-birth rates achieved with ICSI versus conventional IVF using up-to-date national data and included a cost analysis of the two fertilization methods. In cases of non-male factor infertility undergoing PGT-A, the CLBR was 64.7% for ICSI cycles and 69.0% for conventional IVF cycles, with no significant disparity noted after accounting for covariates (adjusted risk ratio, 0.97; 95% CI: 0.93–1.01). No difference was observed in the miscarriage rate (ARR, 0.95; 95% CI: 0.72-1.24) between the two groups (Table 1).

Table 1. Cumulative live-birth and miscarriage rates among day 5 transfers using ICSI vs conventional IVF[24]

Outcome	Without PGT-A			With PGT-A		
	ICSI	cIVF	ARR (95%CI)	ICSI	cIVF	ARR (95%CI)
CLBR (%)	60.9	64.3	0.99 (0.99 - 1.00)	64.7	69.0	0.97 (0.93 - 1.01)
Miscarriage rate (%)	11.3	11.8	1.00 (0.94 - 1.06)	9.0	10.2	0.95 (0.72 - 1.24)

ARR= adjusted risk ratio (adjusted for age, body mass index, number of oocytes retrieved

It was concluded that ICSI does not increase the cumulative live-birth rate compared to conventional IVF method, but increases unnecessary costs in cases of infertility not due to male factors.

Risk of birth defects subsequent to IVF procedures, whether ICSI is involved or not

There is no evaluation of the safety of ICSI for infertility not caused by male factors. Nevertheless, research on male infertility has shown a slight rise in negative consequences for children born through ICSI. The underlying cause of male infertility is usually responsible for these risks. It is not clear how these risks

might be linked to ICSI in patients who do not have male factor infertility. Results from a large population-based research, covering over 308,000 deliveries, with 6,100 originating from assisted reproductive techniques, indicated a heightened risk of significant birth abnormalities following IVF procedures, which was 1.24 times higher after considering potential influencing factors.[25] Among the women receiving fertility treatments, an elevated odds ratio for birth defects was observed solely in those undergoing ICSI (1.57), while the ones undergoing IVF alone did not show the same risk. Nonetheless, the study encompassed participants with both normal and abnormal sperm counts. The well-established fact that men with abnormal semen analyses experience a higher rate of birth defects after IVF is attributed to the chromosomal abnormalities commonly found in such individuals, which may have influenced the outcome of this particular study. Nevertheless, this research adds another element of warning against the inappropriate application of ICSI in every IVF attempt.

A recent study in 2023 utilized a population-based cohort to explore the effects of underlying infertility and fertility treatment on the risks of congenital anomalies (CAs) during the first 24 months of life.[26] This study included 851,984 babies born in New South Wales, Australia from 2009 to 2017, comprising 828,099 singletons and 23,885 multiples. The occurrence of congenital anomalies was 459 per 10,000 singleton births and 757 per 10,000 multiple births. When compared to singleton infants of NC-fertile parents, those conceived through ART showed an increased risk of major genitourinary abnormalities at a rate of 19.0 cases per 10,000 births. The risk was consistent when compared to singleton babies of infertile parents who did not use ART, suggesting that ART continued to present an independent risk. Even when considering parental infertility, couples without male infertility who underwent ICSI had a greater risk of major genitourinary abnormalities, with an absolute risk difference of 47.8 cases per 10000 singleton births ().

The current results confirm earlier studies that pointed to ICSI usage as a separate factor contributing to the occurrence of congenital anomalies, particularly those affecting the genitourinary system.[25,27] Importantly, however, this study was able to adjust for the confounding effect of underlying male factor infertility, hypothesized to be due to genetic alterations causing subfertility in fathers being passed on to the offspring.[28] More than 70% of fertility procedures in the United States and 60% worldwide require the application of ICSI, despite the fact that male infertility is a factor in only about 30% of cases. The findings, along with recent studies, strongly indicate that ICSI is a unique risk factor for congenital abnormalities, especially in the genitourinary system, and should be used selectively for couples with male factor infertility.

Therefore, the safety of ICSI procedure should be evaluated more carefully and ICSI should only be indicated for situations where the chances of IVF success are extremely low, considering both safety and scientific perspectives.

III. CONCLUSION

Thus, with current scientific evidence, for PGT-A cases where the husband has normal sperm quality, routine IVF insemination can be a suitable choice compared to the ICSI method and reduces the cost burden for patients when applying ICSI. ICSI is recommended only when the probability of IVF success is exceedingly low, taking into consideration safety and scientific aspects.

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