Comparison of ICSI and Conventional IVF in Non-Male Factor Patients with Less Than Four Oocytes

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Abstract

Background: We aimed to analyse our clinical results for a particular subgroup of patients with poor ovarian response (POR) in order to clarify if lower number of oocytes is a drawback for proceeding to C-IVF

Materials and methods: In this retrospective study, patient files of all couples (#1733) who underwent oocyte retrieval between January 2017 and December 2019 were reviewed and 191 cases diagnosed with non-male factor infertility in which ≤ 3 cumulus-oocyte-complexes available for fertilisation were analysed. Exclusion criteria were: woman age >42 , patients with a history of previous ART trial, prenatal genetic testing cycles and couples undergoing total cryopreservation for any indication. Three groups were constructed depending on the method of fertilisation and on semen quality as follows: IVF non-male factor (Group 1, $n=77$); ICSI non-male factor (Group 2, $n=65$); ICSI male factor- ICSI/MF $n=49$ according to WHO reference values. Main outcome parameters were: fertilisation rate, implantation rate and live birth rate.

Results: Fertilisation rate per collected COC was significantly higher in group 1 compared to the other two groups (85,68%, 72,58%, 73,33% respectively, $p=0,004$). FR per inseminated oocyte also tended to be higher in group 1 but not reaching a statistically significant level. Both techniques yielded similar implantation rates (20,42%, 28,49%, 23,33% respectively, $p=0,407$) and live birth rates (26,8%, 30,6%, 31,1% respectively, $p=0.643$).

Conclusions: In the presence of normal semen parameters, low egg number is not an indication to perform ICSI. The choice of fertilisation method should be based primarily on semen quality, in combination with the patient's previous history regardless of the ovarian reserve.

Introduction

The first successful in vitro fertilisation (IVF) treatment reported in 1978 was the result of conventional IVF (C-IVF) technique (**Stephoe and Edwards, 1978**). During the following four decades, practitioners and researchers have given continuous effort towards the achievement of higher success rates via improving laboratory set up, cell manipulation techniques and treatment protocols (**Niederberger et al., 2018**). Among all of the innovations delineated heretofore, intracytoplasmic sperm injection (ICSI) can be accentuated as being the second breakthrough, enabling couples with severe male factor infertility to conceive (**Palermo et al., 1992**). In contrary to C-IVF, in which each COC is co-incubated with a certain concentration of sperm suspension, ICSI bypasses the natural barriers of cumulus cells, zona and oolemma which the spermatozoa undergo during the penetration phase of natural fertilisation. Hence ICSI technique provides a single spermatozoon, the chance of achieving the fertilisation process via direct microinjection into a mature oocyte. Despite the lack of evidence based rationale and against the guidelines of international IVF societies (**ESHRE 2016; Boulet et al., 2015**), throughout its twenty five years after its introduction, the utilisation of ICSI increased steadily not only for severe male factor cases, but

for any etiology regardless of age, number of oocytes or background fertility history, currently reaching up to 70-75% in all ART cases both in Europe and in USA. Various publications even proposed and strongly supported the use of ICSI for all infertility etiologies, claiming that it provides higher number of embryos and better clinical outcome (**Orief *et al.*, 2004; O'Neill *et al.*, 2018**). Based on the existing literature, ICSI is more effective in severe male factor only and it does not confer an advantage over C-IVF in other etiologies, rather causing a marginal disadvantage. Furthermore, a potential increase in the risk of certain adverse situations including congenital, chromosomal, epigenetic and functional abnormalities have been attributed to the ICSI per se (**Gjeris *et al.*, 2008; Kohda and Ishino 2013; Kissin *et al.*, 2015; Sandin *et al.*, 2013; Davies *et al.*, 2017**).

The major concern of practitioners as to not preferring C-IVF over ICSI is the fear of encountering total fertilisation failure (TFF). The effect of mass communication media to create a therapeutic illusion among the patients results in social pressure and make it harder for the clinician to inform his/her patient regarding TFF.

We maintain the presumption of clinical practices being solely based on evidence based interventions rather than empirical approach and social coercion. Hence, it is crucial to clarify the rational fundamentals of choosing the right insemination technique. Recently, we published our results comparing the laboratory and clinical outcome of ICSI with those of C-IVF in all non-male factor cases (**Isikoglu *et al.*, 2020**). Since we found similar outcome for both C-IVF and ICSI in the mentioned randomized controlled study, we decided to further analyse our clinical results for a particular subgroup of patients with poor ovarian response (POR) in order to clarify if lower number of oocytes is a drawback for proceeding to C-IVF.

Materials And Methods

This retrospective study was conducted at the Gelecek IVF center, Antalya, Turkey. The study protocol was approved by the institutional review board (IRB no: GTB 20191211). A signed informed consent is routinely obtained from all couples prior to enrolling the ART program.

Study population:

Patient files of all couples (#1733) who underwent oocyte retrieval (OR) between January 2017 and December 2019 were reviewed and 191 cases diagnosed with non-male factor infertility in which ≤ 3 cumulus-oocyte-complexes (COCs) available for fertilization were analysed. Exclusion criteria were: woman age >42, patients with a history of previous ART trial, prenatal genetic testing (PGT) cycles and couples undergoing total cryopreservation for any indication.

Three groups were constructed depending on the method of fertilisation and on semen quality as follows: IVF non-male factor-IVF/NMF (Group 1 n=77), ICSI non-male factor-ICSI/NMF (Group 2 n=65), ICSI male factor- ICSI/MF (Group 3 n=49) according to WHO reference values.

Ovarian stimulation, fertilisation, embryo transfer and pregnancy assessment:

Ovarian stimulation, fertilisation, embryo transfer and pregnancy assessment were done as described elsewhere (**Isikoglu *et al.*, 2020**). Briefly, initial daily gonadotropin dose was defined as 225-300 IU/day depending on the body mass index and the previous history of the patients. In our clinical protocol, we prefer hMG to FSH-only preparations in POR patients. Daily GnRH antagonist (Cetrotide® 0,25 mg, Merck/Germany) injections commenced once the leading follicle reached 14 mm in diameter. Final oocyte maturation was induced with 250 µg recombinant hCG (Ovitrelle, Merck/Germany). Transvaginal ultrasound-guided OR was done 36 hours after hCG injection. Density gradient technique was used routinely for semen preparation. In C-IVF group, the COCs were placed in each well of a four-well dish with Fertilisation Medium® (Cook, Australia) and inseminated with sperm suspension containing 100.000/ml motile spermatozoa per/COC whilst the COCs in ICSI groups were denuded to examine oocyte maturation and metaphase II (MII) oocytes were inseminated within four hours after oocyte retrieval by ICSI.

The eligibility to be enrolled in C-IVF was based on our clinical cut off index for semen quality (namely “C-IVF index” defined as total progressively motile sperm with normal morphology count ≥ 100.000). ICSI was used in all cases under this threshold. For couples with an index above this limit, the decision to perform C-IVF or ICSI depended on the decision of the couple in addition to the clinical approach of the IVF practitioner. Prior to this decision, couples were informed about the pros and cons of both methods.

Embryos were graded between 1 and 4 at cleavage stage based upon gross embryo morphology as described by Balaban et al (**Balaban *et al.*, 2001**). All embryo transfers were performed at cleavage stage on day three after OR.

Luteal phase was supported with vaginal administration of 200 mg progesterone capsules three times a day (Progestan 200 mg®, Koçak Farma/Turkey) starting from the day after OR and continued until a negative pregnancy test or 8th gestational week.

Main outcome parameters were: fertilisation rate, implantation rate and live birth rate.

Continuous variables were defined with (mean $\pm$ standard deviation) or median (quartiles), and compared between the groups with Independent Sample t Test (if the data size is enough in each group) or Mann Whitney U Test (if the data size is not enough in each group) test based on distribution characteristics. Categorical variables were defined with numbers and percentages and compared with chi square test or derivatives as appropriate. A two sided p value <0.05 was considered significant.

Results

Baseline characteristics and ovarian reserve of the patients were similar for the three groups (**Table I**). Anti Müllerian Hormone levels were not analysed since it was not a part of the routine infertility work up during the study period.

Table I: Baseline characteristics and ovarian reserve of the patients (**BMI**: body mass index, **AFC**: Antral follicle count)

	IVF-NMF	ICSI-NMF	ICSI-MF	p value
No of patients	77	65	49	NA
Patients' age	36,01±4,63	35,69±4,98	35,15±5,05	0,62
Husbands' age	38,56±6,12	37,89±7,54	37,96±6,11	0,81
BMI	24,52±4,89	24,23±3,99	25,83±4,93	0,16
FSH	11,28±6,17	12,25±13,11	9,20±5,70	0,52
AFC	4,44±2,43	4,47±2,58	5,23±3,51	0,26

When etiologic reasons were compared, percentage of male factor infertility cases was significantly higher in group 3 as expected. Percentage of ovulatory factor was lower in group 3 which may be due to low number of patients in study groups. The frequency of all other etiologies were similar for all groups (**Table II**).

Table II Etiology of infertility

	IVF-NMF	ICSI-NMF	ICSI-MF	p value
Tubal factor	14	8	3	0,14
Ovulatory factor	61	52	29	0,02
Endometriosis	7	4	3	0,75
Male factor	0	0	49	0,00
Unexplained	3	1	0	0,31

Stimulation characteristics (**Table III**), laboratory parameters (**Table IV**) and clinical outcome variables for the three groups (**Table V**) are shown below. Duration of COS was significantly lower in group 3 compared to group 1. Fertilisation rate per collected COC was significantly higher in group 1 compared to the other two groups. FR per inseminated oocyte also tends to be higher in group 1 but not reaching a statistically significant level.

Table III Stimulation characteristics

	IVF-NMF	ICSI-NMF	ICSI-MF	p value
Duration of COS (days)	8,78±1,89	8,12±1,92	7,78±1,59	0,008
Total gonadotropin dosage	2201,95±735,73	1990,22±704,24	1922,45±626,48	0,06
Peak estradial in pg/mL	508,64±339,87	448,34±248,76	499,75±253,12	0,63

Table IV: Laboratory variables (**FR:** Fertilisation rate **COC:**Cumulus oocyte complex)

* Top quality embryos are the sum of grade 1 and grade 2 embryos

	IVF-NMF	ICSI-NMF	ICSI-MF	p value
COC (no)	2,17±0,77	2,42±0,73	2,33±0,8	0,150
FR per collected COC (%)	85,68%	72,58%	73,33%	0,004
FR per inseminated oocyte (%)	85,68%	78,46%	78,91%	0,920
Top quality embryos (%)*	83,80%	85,48%	87,41%	0,791
Embryos transferred (no)	1,35±0,48	1,34±0,48	1,27±0,45	0,610
Total Fertilisation Failure (%)	4 (5,2%)	2 (3,1%)	4 (8,2%)	0,480
Cancelled ET (%)	3 (3,9%)	3(4,6%)	4 (8,2%)	0,850

In three out of four TFF cases in C-IVF group, rescue ICSI was carried out the day after oocyte retrieval. Although normal fertilisation and embryo transfer was achieved in all three cases, only one of these patients achieved pregnancy, resulting in healthy singleton delivery.

Table V Clinical outcome variables

	IVF-NMF	ICSI-NMF	ICSI-MF	p value
IR (%)	20,42%	28,49%	23,33%	0,407
CPR/OPU (no and %)	28,6% (22/77)	36,9%(24/65)	32,7%(16/49)	0,571
CPR/ET (no and %)	31% (22/71)	38,7%(24/62)	35,55(16/45)	0,643
Live birth rate (no and %)	26,8%(19/71)	30,6%(19/62)	31,1% (14/45)	0,514
Chemical abortion/ET (no and %)	8,45% (6/71)	4,83% (3/62)	2,22% (1/45)	0,346

Discussion

Our results suggest that whilst fertilisation rate per collected oocyte is better via C-IVF, both techniques yield similar implantation rates, live birth rates and miscarriage rates in patients with less than four COCs. Even though fertilisation rate per inseminated oocyte appearing higher in IVF-NMF group compared to ICSI groups it was not statistically significant.

Based on the registry of European IVF Monitorization Consortium (EIM), in European countries, the proportion of ICSI in total Assisted reproductive technology (ART) cycles has increased steadily between 1999 and 2006 and drew a plateau thereafter at a level of nearly 70% (**ESHRE 2016**). Data from United States show a similar pattern that ICSI use has been shown to have increased from 36.4% in 1996 to 76.2% in 2012 for all etiologic subgroups with the largest increase in non-male factor infertility cases (**Boulet et al., 2015**). Contemplating the global situation, the ICSI/C-IVF ratio is lowest in Asia as 1.4, this is followed by Australia, New Zealand and Saharan Countries as 2 and highest in Middle East, reaching up to more than 60 (**Dyer et al., 2016**). Despite ICSI having a high popularity in the field of reproductive treatments, both American Society of Reproductive Medicine (ASRM) and ESHRE guidelines inadvised routine implementation of ICSI for all oocytes and suggest to reserve it for the male factor infertility cases or for patients with a history of TFF in previous attempts (**Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology. Electronic address: asrm@asrm.org. Intracytoplasmic sperm injection (ICSI) for non-male factor indications: a committee opinion. Fertil Steril. 2020 Aug;114(2):239-245. doi: 10.1016/j.fertnstert.2020.05.032. Epub 2020 Jul 9. PMID: 32654822.ASRM 2012; Good Clinical Treatment ESHRE**). Based on the extended registry of Society of Assisted Reproductive Technology Clinical Outcome Reporting System (SART CORS), considering the non-male factor infertility, live birth rates are higher via C-IVF compared to ICSI (SART 2016). A recent meta-analysis of four randomized controlled studies showed ICSI having no advantage over IVF in fertilisation rate, clinical pregnancy rate and implantation rate. Analysis of twenty two cohort studies in the same systematic review demonstrated a significantly higher implantation rate and live birth rate in favor of C-IVF (**Geng et al., 2020**). The findings of the largest cohort study so far involving nearly 1.4 million ART cycles were in line with this meta-analysis. In their study analysing the rich database of Human Fertilisation and Embryology Authority (HFEA), the authors assessed reproductive outcomes following C-IVF and ICSI in POR patients with normal semen parameters. The clinical outcome of all ovarian response categories were also compared in this study. It is noteworthy that the total fertilisation rates were similar for both techniques in POR patients (17.3% vs 17% respectively). Although failed fertilisation rate was statistically higher for IVF cycles (4.8%) compared to ICSI cycles (3.2%) in the entire cohort, this statistically significant finding may have reflected the very large sample size and may lack clinical relevance. Considering all ovarian response categories, the authors found no benefit in clinical pregnancy rate and live birth rate with ICSI in non-male factor patients (**Supramaniam et al., 2020**). In another retrospective multicenter study conducted in 15 European tertiary centers and involving nearly five thousand patients having C-IVF or ICSI for non-male factor infertility, similar live birth rates were

found for C-IVF and ICSI in poor, suboptimal, normal and high responders. This suggests that the number of COCs retrieved is not a determinant for the selection of the insemination technique in non-male factor cases (**Drakopoulos et al., 2019**).

Then, what could be the rationale for clinicians and embryologists to dramatically overuse ICSI in non-male factor infertility against the recommendations of guidelines and evidence based data?

There are two main reasons for IVF practitioners to prefer ICSI irrespective of the infertility etiology, the first reason is the concern for possible TFF with C-IVF, whilst the second being the fictive belief that C-IVF results in lower fertilized oocytes. Thus, main point of ICSI supporters is maximizing the number of embryos and minimizing the risk of complete failure of fertilisation. Indeed existing database encourages ICSI as not preventing TFF in patients with non-male factor infertility and TFF rates being similar with both methods (SART 2016; Bhattacharya et al., 2001; **Gennarelle et al., Kim et al., 2014**). The paramount advantage of ICSI over C-IVF is the higher fertilisation rate of inseminated oocytes due to the direct injection of a single spermatozoon into a mature oocyte instead of co-incubation of COCs with a certain concentration of sperm cells, being the technique in C-IVF. But it is worth emphasizing that this superiority is valid if the fertilisation rates of both techniques are compared per inseminated oocyte while fertilisation rate per collected oocyte is generally reported to be either similar or higher via C-IVF (Bhattacharya et al., 2001; **van Rumste et al., 2004; Li et al., 2018**). The superiority of C-IVF in fertilisation rate per all collected COCs is theoretically explicable through the fact that immature oocytes may have the chance of final maturation process in in vitro conditions by co-insemination with sperm suspension with their cumulus cells intact. The confusion created by the theoretical possibility of TFF and fictive thought of higher fertilisation rates result in the hesitancy of performing C-IVF in non-male factor cases and in cases with borderline semen parameters. Consequently, it is straightforward that the thought of ICSI results in better fertilisation compared to C-IVF is nothing more than a therapeutic illusion.

In the present study we encountered similar TFF rates in three groups. In our previous study comparing C-IVF with ICSI in normo responder non-male factor cases (**Isikoglu et al., 2020**), we confronted total fertilisation failure in ten cases. All of these were in C-IVF group. When we further analysed the variables of TFF group in that study and compared with those of non-TFF group, all semen variables except morphology were without a remarkable difference. We then proposed that TFF must have been due to our usage of stricter criteria for the definition of non-male infertility compared to other studies. Thereafter, we modified our clinical protocol and set a higher cut off value (total progressively motile sperm with normal morphology count ≥ 100.000) for eligibility to enroll C-IVF in the process of eliminating the risk of TFF. Indeed, it is well documented that conventional or computerized semen analysis cannot predict the fertilizing capacity of a semen sample (**Guzick et al., 2001**). That is why neither ESHRE Special Interest Group of Embryology nor Alpha Scientists in Reproductive Medicine present a certain cut off semen index for proceeding to C-IVF (**De los Santos et al., 2015; ESHRE and Alpha Scientists 2017**). Instead, it is recommended that while choosing the insemination method, laboratories should develop and apply their own criteria depending on their laboratory data and clinical experience. The modification of our C-IVF

index by time should be regarded as a continuous improvement of the process, aiming to increase its efficiency.

While there are no additional maneuvers in case of TFF after ICSI, there is an alternative procedure to rescue the unfertilized oocytes after C-IVF, which is the re-inseminating of the oocytes via ICSI that have not fertilized with C-IVF. According to some publications, this procedure –known as rescue ICSI or late ICSI- may cause high rates of polyploidy and arrest at early developmental stages of the embryos **(Tsirigotis et al., 1995)** while some others report more encouraging results like similar pregnancy rates and congenital defects when compared to C-IVF or ICSI **(Huang et al., 2015)**. Rescue ICSI has been reported to be performed either 6 h after insemination (early rescue) or the day after oocyte retrieval (late rescue) the former giving more promising results. A systematic review covering data of twenty one years published between 1992 and 2013 reported a pregnancy rate of 17.8% per embryo transfer with rescue ICSI **(Beck-Fruchter et al., 2014)**. In the present study, we encountered TFF in four cases in C-IVF group and late rescue ICSI was carried out in three of them. Although normal fertilisation was achieved in all three cases, only one patient achieved pregnancy, resulting in healthy singleton delivery.

POR patients construct a special group lying in the worse part of the ovarian response spectrum. There are a scarce number of studies in the literature comparing both techniques in poor responders and in advanced age patients. In their retrospective study including more than six hundred patients with no more than five oocytes, Liu et al found higher implantation rates and cumulative live birth rates in C-IVF arm (15% vs. 8% and 15% vs. 6% respectively) **(Liu et al., 2018)**. In another study, 1305 patients with an oocyte number between four to one were analysed retrospectively. Implantation rates, clinical pregnancy rates, live birth rates, cumulative pregnancy rates and cumulative live birth rates were found to be comparable between C-IVF and ICSI groups **(Guo et al., 2018)**. In another retrospective study with nearly 250 patients, laboratory and clinical results of C-IVF results were found to be comparable to those of ICSI even in patients with a single oocyte **(Sfontouris et al., 2015)**. Live birth rates appeared to be similar (11.9 vs 9.6%) when both techniques are compared in women aged ≥ 40 **(Tannus et al., 2017)**. Analysing data of more than six hundred patients, Gennarelli et al reported similar results for patients aged ≥ 40 **(Gennarelli et al., 2019)**.

In addition to higher fertilisation rate per inseminated oocyte and the non-inferiority of C-IVF regarding the laboratory and clinical outcome parameters **(Li et al., 2018; Grimstad et al., 2016)**, there are other prominent advantages of C-IVF including safety, cost effectiveness and feasibility. As explained in detail in our previous study **(Isikoglu et al., 2020)**, C-IVF is much more cost effective creating a striking advantage for the IVF clinic and the infertile couple. This point is generally overlooked due to the lack of studies regarding cost effectiveness of the procedures. ICSI is also much more time consuming necessitating to stay in the laboratory for hours depending on the number of oocytes and the number of the OR procedures while it takes just a few minutes to accomplish C-IVF. In summary, ICSI causes extra burden both for the oocytes and the laboratory staff while not promising better success rates in non-male factor infertility cases. Moreover, unlike ICSI, C-IVF does not depend on hand skills, hence there is an

exponentially rising learning curve enabling an unexperienced embryologist to perform it as easily and efficiently as an experienced one.

In addition to the above listed disadvantages of ICSI one should keep in mind that during ICSI process, the spermatozoon is not naturally selected by the cross-talk between a cohort of sperm cells and the COC. In contrast to C-IVF, natural barriers to fertilisation are by-passed mechanically by microinjection of the artificially chosen spermatozoon directly in the cytoplasm of the oocyte. It may easily be speculated that by-passing this natural selection step may render the embryo some potential risks including genetically transmitted disorders. Although ICSI supporters claim that the long term medical and reproductive health of ICSI children seems reassuring (O'Neill et al., 2018), there are many contradictory studies reporting a possible association between ICSI and increased congenital, perinatal, developmental, and reproductive health problems including chromosomal abnormalities, congenital heart disease, mental retardation, autism, imprinting disorders and prolonged stay in neonatal intensive care unit (Gjerris et al., 2008; Kohda and Ishino 2013; Kissin et al., 2015; Sandin et al., 2013; Davies et al., 2013). Considering that the oldest ICSI individuals are in their late twenties, we cannot be sure about the long term potential risks which a less natural procedure may result in.

Retrospective design, small number of patients in the groups are the limitations of the present which necessitates careful interpretation of the findings.

Conclusion

In the presence of normal semen parameters, low egg number is not an indication to perform ICSI. Since there is not any convincing reason for using a technique with relative disadvantages for a group of patients without a strict indication, C-IVF should be the choice of technique in properly selected non-male factor infertility cases even if the number of COCs is limited. Indeed ICSI is only an alternative to C-IVF which is the first line and more natural available option. Therefore, the choice of fertilisation method should be based primarily on semen quality, in combination with the patient's previous history regardless of the ovarian reserve.

Declarations

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