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GYNECOLOGIC ENDOCRINOLOGY AND REPRODUCTIVE MEDICINE

When to do intracytoplasmic sperm injection: a prospective comparison

Grace Younes[1] · Samer Tannus[1] · Weon-Young Son[1] · Michael H. Dahan[1]

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Abstract

Purpose The purpose of the study was to assess the fertilization rate and embryo development in sibling human oocytes after split insemination in patients with and without isolated teratozoospermia.

Methods A prospective cohort study at a university affiliated reproduction center was performed. Hundred and three patients during the time periods 01-2013 to 12-2015 had split insemination ordered for their first IVF cycle. The primary outcome measured was fertilization rate. Secondary outcomes were the number and quality of embryos.

Results Mature oocytes at the time of collection were assigned as follows: 558 to IVF and 556 to ICSI. An additional 48 immature oocytes matured while awaiting spontaneous fertilization with IVF for a total of 606 in that group. The study group of normal strict sperm morphology ≤ 4 included 61 patients, and the control group included 42 patients with normal strict sperm morphology > 4. ICSI was statistically favored over IVF only in cases with normal strict sperm morphology ≤ 4%. There was a higher fertilization rate in ICSI compared to IVF (74.4% vs. 38%, $p < 0.0001$), a higher number of day 2 (4 ± 3.4 vs. 2.4 ± 2.7, $p < 0.0001$), day 3 (4 ± 3.4 vs. 2.2 ± 2.7, $p < 0.0001$) and day 5 embryos (2.2 ± 2.6 vs. 1.2 ± 2, $p = 0.001$), and they were of better quality; however, it did not reach significance ($p = 0.062$). A similar advantage for ICSI was seen in a subgroup of unexplained infertility with normal strict sperm morphology > 4%.

Conclusions In conclusion, in couples with normal strict sperm morphology ≤ 4%, there is an advantage of ICSI over IVF in terms of fertilization rate, quantity and quality of cleavage stage embryos and blastocysts. Based on the results, ICSI seems reasonable as a first-line treatment in patients with normal strict sperm morphology ≤ 4%, as well as in patients with unexplained infertility.

Keywords Teratozoospermia · Fertilization failure · Sibling oocytes · Split insemination · ICSI

Introduction

Sperm classification on the basis of morphologic criteria was proposed by Kruger et al. as a predictor for fertilization potential [1]. Subsequent modifications to this classification system led to the strict Kruger/Tygerberg criteria which aimed to provide better correlations with functional tests and fertilization outcomes. Later publications demonstrated that a relationship existed between IVF outcomes and the degree of morphological normality of the spermatozoa, significantly reduced fertilization rates and an increased incidence of failed fertilizations, and lower implantation rates and pregnancy rates in individuals with < 5% normal sperm morphology [2–6].

Dubey et al. concluded that among couples undergoing IVF preimplantation genetic diagnosis cycles, men with normal sperm morphology were statistically more likely to have euploid embryos, higher implantation rates and clinical pregnancy rates per cycle and per embryo transfer, than those with teratozoospermia [7]. In spite of the results presented above, the impact of isolated teratozoospermia is still controversial, as studies have shown conflicting results concerning the effect of teratozoospermia on fertilization, pregnancy rates and quality of embryos [8–11]. Robinson et al. showed that the majority of patients with normal sperm morphology < 5% achieved good fertilization rates with IVF, without ICSI, as long as the sperm concentration and motility were within normal range [11].

✉ Grace Younes
graceyounes@gmail.com

[1] MUHC Reproductive Centre, 888 Boul de Maisoneuve East #200, Montreal, QC H2L 4S8, Canada

Due to the conflicting conclusions regarding the effect of teratozoospermia on IVF outcomes, and fear of fertilization failure especially in the first IVF cycles, some clinicians adopted the ICSI–IVF insemination split procedure in sibling oocytes to help eliminate fertilization failures. Decisions concerning the treatment technique (conventional IVF, ICSI or both) in teratozoospermia is still, however, an area of considerable debate, as the advantages of ICSI when compared to IVF are uncertain.

While some studies have shown lower fertilization rates with conventional IVF in the presence of teratozoospermia [11–13], others have shown no advantage of ICSI on IVF as long as the sperm concentration and motility were within the normal range according to World Health Organization (WHO) standards [14, 15]. However, these studies were based on the 1990 and the 1999 WHO standards, respectfully, which have now decreased levels with the publication of the 2010 WHO semen evaluation manual. It is unknown if the results found in these two studies apply to currently normal semen analysis results.

Most studies examining the benefit of ICSI over IVF in teratozoospermia failed to use sibling oocytes, and are therefore open to substantial biases. Two prospective studies on sibling oocytes with isolated teratozoospermia have found a higher fertilization rate with ICSI than with IVF and lower complete fertilization failure, but no advantage on embryo quality [12, 13], Fan et al. however, found no such difference [16]. On the other hand, another study showed a higher incidence of cytoplasmic fragmentation of the embryos and lower number of high-quality embryos after IVF when compared with ICSI [17].

Until 2013, all patients with normal sperm morphology ≤ 4% had 100% ICSI ordered. Lack of definite advantage of 100% ICSI, but fearing fertilization failure, 50% ICSI was ordered instead in all of these patients. The primary objective of this study was to compare the fertilization rate in sibling oocytes fertilized by 50% ICSI and 50% IVF in patients with and without isolated teratozoospermia.

Materials and methods

This study included all patients treated by a single physician for their first IVF cycle with normal sperm morphology ≤ 4% ($N = 61$) on their baseline semen analysis from January 1, 2013, to December 31, 2015, at the reproductive unit of the McGill University Health Centre in Montreal, Canada. An additional control group with normal sperm morphology greater than 4% was also offered split insemination. These subjects included diagnoses of unexplained infertility ($N = 29$), tubal factor infertility ($N = 5$), anovulation ($N = 5$) and decreased ovarian reserve ($N = 3$). Patients were prospectively enrolled in the study database. All patients were consented for 50% ICSI 50% IVF, and the benefits and risks of each were discussed. Patients were also offered either 100% ICSI or 100% IVF where appropriate. The IVF consent was signed by each patient, with ordering of 50% ICSI.

Split insemination of sibling oocytes was ordered for these patients during their first IVF cycle. All patients were recorded prospectively in the database when their IVF cycle was ordered, and outcomes were tracked. Subsequently, institutional review board approval was obtained to evaluate the database. All patients were included only once, for their first IVF cycle. Subjects were included with low and normal sperm morphology to understand the role that strict morphology plays in fertilization.

During the study time period, 140 patients meeting the criteria had IVF ordered and recorded prospectively, 124 underwent IVF treatment during the time frame, and 103 of them had split insemination performed (50% ICSI, 50% IVF approximately) and were included in the study. Sixty one patients had normal strict sperm morphology ≤ 4%, and 42 had normal strict sperm morphology of > 4%. Twenty patients had only ICSI performed on all oocytes due to poor sperm quality incompatible with IVF alone as determined by the embryology laboratory at the day of insemination. One patient had 100% IVF performed due to exclusively immature oocytes being collected (Fig. 1). All IVF cycles were provided free of charge to the patients, under the Quebec health insurance plan.

Relevant clinical data extracted included: female and male age**,** body mass index (BMI) within 3 months of infertility treatment, duration and etiology of infertility, baseline ovarian reserve markers within 3 months of treatment, baseline semen analysis, semen analysis on the day of oocyte retrieval in the specimen used for insemination, type of treatment protocol used (microdose flare, a fixed antagonist, and a midluteal long agonist protocol), monitoring parameters during the treatment, laboratory and clinical outcomes. For more information on the IVF protocols used please see Ref. [18].

Baseline sperm analysis was performed within an hour from obtaining the sample, after incubation at 37 °C and liquefaction. The sperm was analyzed by a sperm analyzer (CASA-system; HTM-IVOS, version 12.3. HAMILTON-THORNE biosciences, MA, USA) with intra- and interassay coefficients of variation < 10%. The concentration and motility of the sperm were determined by CASA. As for the sperm morphology evaluation, a smear was prepared from 5 to 20 µl semen and stained with "Siemens Diff Quick stain kit" (VWR, SIEMENS HEALTHCARE LTD, CA), a 1000 × magnification was used, and the morphology of at least 200 sperm cells was determined for each sample. All slides were examined by the same technician. In all cases, analysis of morphology was repeated twice to confirm the

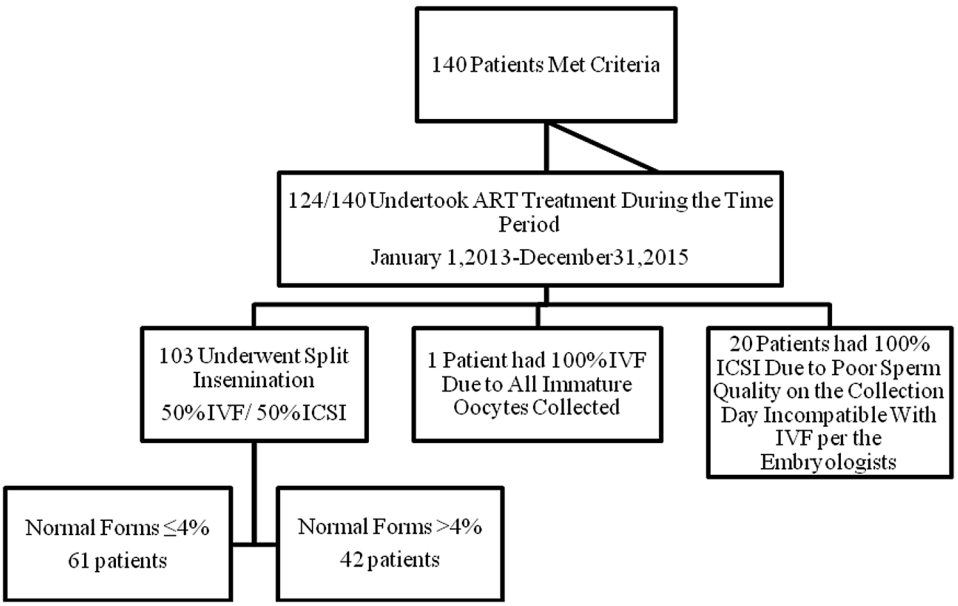

Fig. 1 Patients enrollment and distribution

result. In the case of a discrepancy, the average morphology was calculated.

Folliculogenesis was monitored during the controlled ovarian stimulation treatments, using serial transvaginal ultrasound scans (GE Volouson 730 pro, General Electric Corporation, USA) and serum estradiol concentrations. Ovulation triggering was ordered when at least two 18 mm follicles were seen, and oocyte retrieval was performed 36 h thereafter.

Oocyte retrieval was performed transvaginally under local anesthesia, and the retrieved oocytes were then divided equally to group A and B by a first embryologist in a blinded fashion. A second embryologist then performed ICSI on one of the two groups. The two embryologists were not allowed to communicate about what oocytes were assigned to the groups or which groups were selected for IVF and ICSI. As such, the embryologists were blinded to what was in each group.

On the day of oocyte retrieval, a fresh sperm sample was obtained and prepared using the density gradient (PureSperm 100, Nidacon International, Sweden) method for oocyte insemination.

For ICSI, cumulus stripping was performed 2 h after oocyte retrieval. MII oocytes were then inseminated by ICSI at least 1 h after the cumulus cell stripping. Only mature oocytes were injected in the ICSI group, while immature oocytes identified at the time of oocyte stripping were discarded. Some MI oocytes continued maturation and fertilized after identification of maturation 2 h after the collection in the IVF group. This phenomenon has been well described [19, 20]. However, this did not happen in the ICSI group, having discarded all immature oocytes at the time of oocyte stripping. The clinic has extensive experience performing in vitro maturation (IVM) and has published guidelines on how to determine the maturity of unstripped oocytes at collection [21]. Determining maturity at the time of oocyte collection in IVM is hampered due to the condensed cumulus cells. The expanded cumulus cells make identification of maturity level during an IVF collection easier than at IVM, with the technique otherwise being the same. We are unsure if this technique has been published for IVF previously.

Our center is one of the premier IVM laboratories in the world. This experience with IVM results in the ability to identify mature unstripped oocytes accurately (which is required to be able to successfully perform IVM). The maturity is assessed based on two techniques that can be used: the sliding method or the spreading method [21], which enables determining the number of MII oocytes assigned to the conventional IVF group. These two techniques have been previously published and validated [22, 23].

For conventional IVF, the oocyte–cumulus–corona complexes were inseminated with 100,000/ml motile spermatozoa per insemination dish (Oodaef® 4 well dish, Denmark), containing three to five eggs. For ICSI, sperm was selected based on the morphology and motility and ICSI performed with an injection needle (Origio, USA) under 200 × magnification using an inverted microscope (Nikon eclipse Ti, Japan).

Fertilization was assessed in both groups 16–18 h after insemination for the appearance of two distinct pro-nuclei and two polar bodies. The fertilization rate was determined per MII oocyte. The zygotes were cultured in 20 µl droplets of single culture global medium (Global total, LifeGlobal, USA) under mineral oil (Global, LifeGlobal, USA) and incubated in an atmosphere of 6.0% CO_2, 5% O_2 and 81.0% N_2.

Luteal support consisted of micronized progesterone given vaginally, started on the day of oocyte retrieval, and continued if pregnant for 12 weeks of pregnancy; for more explanation please refer to our previous article [19]. Embryo transfer day was determined based on the quantity and quality of embryos on days 3 or 5. The best quality embryos from either group IVF or ICSI was selected and transferred; other good-quality embryos were vitrified.

The assessment of embryo quality was as follows: cleavage embryos were defined as good quality (Grade 1 or 2) if they had four cells on day 2 and/or seven or eight cells on day 3, contained < 20% fragmentation, and exhibited no apparent morphological abnormalities. Poor-quality embryos included fair-quality (Grade 3) embryos, which had only two cells on day 2, three to five cells on day 3 and/or 20–50% fragmentation; and Grade 4 embryos included < 3 cells by day 3 and > 50% fragmentation. Embryos showing blastomere multi-nucleation, poor cell adhesion, uneven cell division and cytoplasmic abnormalities were defined as low quality[24]. On day 5, embryos were scored for blastocyst formation and graded according to the size of the blastocyst, the assessment of the inner cell mass (ICM) and trophectoderm development (≥3BB) [25]. Good-quality embryos (Grade 1 or 2) were defined as those where at least the blastocele completely filled the embryo (3), the ICM was loosely grouped with several cells (B) and the trophectoderm had a few cells forming a loose epithelium (B). Lower than 3BB quality embryos on day 5 were defined as poor-quality embryos (Grade 3 or 4).

All patients underwent a transfer of one or two embryos, on day 3 or day 5. Criteria to grow to day 5 was at least three good-quality day 3 embryos. All non-transferred embryos were cultured until day 5 and assessed and then frozen as blastocysts.

βHCG levels were tested 16 days after oocyte retrieval. Clinical pregnancy was defined as positive intrauterine gestational sac seen on US 2 weeks after a positive blood test for βHCG.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences 23.0 (SPSS INC, Chicago IL). Paired samples statistics were used for laboratory outcome comparisons. Chi squared tests were used for pregnancy outcomes. Since the study used paired samples from each patient, the subject automatically controls for confounding factors. Data are presented as mean ± standard deviation or percentages. Two-sided p values ≤ 0.05 were accepted as statistically significant. Human Subjects Committee approval of the study was obtained.

Results

Hundred and three patients had split insemination performed during their first IVF cycle and were included in the study (Fig. 1). Sixty-one patients in the study group had normal strict sperm morphology ≤ 4%, and 42 patients as a control group had normal strict sperm morphology > 4%. The mean age for the male partners and females were comparable in both groups. Average antral follicle count was 18.9 ± 12.1 vs. 16.8 ± 10.2, respectively, and baseline serum FSH levels were 7.3 ± 2.7 vs. 7.3 ± 2.2 IU/l. The duration of infertility for the couples was lower in the ≤ 4% group (26.4 ± 18.3 months vs. 38.7 ± 34.3 months, $p = 0.046$) (Table 1).

In the ≤ 4% group, as compared to the > 4% normal strict sperm morphology group, baseline semen volumes were similar, while baseline semen concentration, motility and normal morphology were higher in the > 4% group on average (Table 1).

The sperm morphology was not evaluated on the day of oocyte collection. An average time of 4.7 months was passed between the test and oocyte collection (4.7 ± 3.6, median 3.5, min 1, max 12). An average time of less than 6 months is likely acceptable for decision making in IVF treatments. Changes in sperm parameters can happen over time; however, the change should be minimal over such a short time.

The average concentration and motility on the day of oocyte collection were comparable between the ≤ 4, and > 4% groups (Table 1)

At collection, 1445 oocytes were obtained, and 1114 were mature oocytes: 558 were assigned to IVF and 556 to ICSI in a blinded fashion. An additional 48 immature oocytes underwent spontaneous maturation in the IVF group for a total of 606 mature oocytes undergoing IVF. The mean number of oocytes collected per patient was 14.0 ± 8.6 (range 3–46). The mean numbers of mature oocytes in the conventional IVF and ICSI groups were 5.9 ± 4.2 and 5.4 ± 3.8, respectively ($p = 0.04$), favoring IVF.

When evaluating all patients, the fertilization rate in the ICSI group was higher than in the conventional IVF (74.28% vs. 47.02%, $p < 0.0001$). Complete fertilization failure was reported in 24 patients in the IVF and 6 patients in ICSI ($p < 0.0001$). The best embryo was selected for transfer by the embryologists, and the remaining good-quality blastocysts were vitrified for future use.

The cohort was stratified based on normal strict sperm morphology and ICSI vs. conventional IVF compared in each of these groups. Based on the baseline normal sperm

Table 1 Demographics

	All study patients ($N=103$)	Normal forms ≤ 4% ($N=61$)	Normal form ≥ 5 ($N=42$)	p	Normal forms ≤ 4% WHO 2010 criteria	Normal forms ≤ 4% WHO 1999 criteria	p	Unexplained infertility
Female age (years)	34.4 ± 4.3	34.1 ± 4.1	34.8 ± 4.6	0.46	34.5 ± 3.9	33.9 ± 3.6	0.52	35.8 ± 4.2
Male age (years)	37.9 ± 6.1	37.9 ± 6.3	38.2 ± 5.8	0.84	38.6 ± 6.6	37.8 ± 5.9	0.61	38.6 ± 26.5
Duration of infertility (months)	31.1 ± 25.9	26.4 ± 18.3	38.7 ± 34.3	**0.046**	28.3 ± 18	27.9 ± 18.7	0.4	31.6 ± 26.5
Antral Follicle count	18.2 ± 11.4	18.9 ± 12.1	16.8 ± 10.2	0.39	18.8 ± 13.4	18.4 ± 13.2	0.4	16.6 ± 11.4
Basal serum FSH (IU/ml)	7.3 ± 2.5	7.3 ± 2.7	7.3 ± 2.2	0.88	7.7 ± 2.9	7.9 ± 3.1	0.78	7.2 ± 2.3
Maximal stimulated serum estradiol (pmol/l)	7571.6 ± 3889	7473 ± 3987	8005.1 ± 3856	0.51	7185.2 ± 4218.8	7691.6 ± 4876.9	0.64	7574 ± 3354.2
Maximum endometrial thickness (mm)	10.4 ± 2.1	10.5 ± 2.2	10.1 ± 1.9	0.38	10.4 ± 2.2	10.3 ± 2.1	0.85	10.1 ± 1.6
Total number of eggs collected per cycle	14.2 ± 8.6	13.9 ± 9.1	14.8 ± 8.1	0.6	13.1 ± 9.3	14.3 ± 10.7	0.6	12.4 ± 4.2
Baseline semen volume (ml)	2.81 ± 1.26	2.9 ± 1.3	2.5 ± 1.2	0.09	2.9 ± 1.2	3.4 ± 1.1	0.08	2.5 ± 1.2
Baseline mean sperm concentration (million/ml)	57.52 ± 40.04	42.4 ± 27.3	78.2 ± 44.7	**< 0.0005**	52.7 ± 26.6	50.5 ± 19.9	0.71	68.8 ± 34.4
Baseline mean sperm motility (%)	66.48 ± 22.76	57.7 ± 23.4	79.7 ± 15.4	**< 0.0005**	68.9 ± 16.4	70.3 ± 14.1	0.72	75 ± 15.6
Baseline percentage of normal forms (%)	3.85 ± 1.97	2.6 ± 1.2	5.9 ± 1.0	**< 0.0005**	3.0 ± 1.1	3.1 ± 1	0.71	4.7 ± 1.8
Treatment Mean sperm concentration (pre processing)	36.9 ± 26.5	20.6 ± 2.6	34.4 ± 5.5	0.33	36.4 ± 20.7	36.7 ± 21.5	0.9	38.4 ± 25.6
Treatment mean sperm concentration (post processing)	41.5 ± 26.9	24.1 ± 3.1	30.2 ± 4.9	0.4	41.3 ± 22.7	45.7 ± 23.9	0.4	52.4 ± 27.6
Treatment mean sperm motility (pre-processing)	35.9 ± 18.5	17.8 ± 2.3	19.3 ± 3.1	0.26	37.7 ± 16.4	39.2 ± 17.8	0.7	40.2 ± 18.2
Treatment mean sperm motility (post-processing)	80.9 ± 13.9	12.9 ± 1.6	13.3 ± 2.2	0.12	83.5 ± 8.4	83.5 ± 7.9	1	82.7 ± 11.7

Values that are statistically significant are given in bold

morphology, the group was stratified into ≤ 4% and ≥ 5% normal sperm morphology. ICSI was statistically favored over IVF only in cases with normal sperm morphology ≤ 4% (Table 2). There was a higher fertilization rate in ICSI compared to IVF (74.4% vs. 38%, $p < 0.0001$), a higher number of day 2 (4 ± 3.4 vs. 2.4 ± 2.7, $p < 0.0001$), day 3 (4 ± 3.4 vs. 2.2 ± 2.7, $p < 0.0001$), and day 5 embryos (2.2 ± 2.6 vs. 1.2 ± 2, $p = 0.001$), and they were of better quality; however, it did not reach statistical significance ($p = 0.062$) and there were 18 cases of failed fertilization with IVF in this study group. Comparison of fertilization rates, embryo quantity and quality were not different when evaluating couples with normal sperm morphology ≥ 5%.

Subsequently, the data were classified based on isolated teratozoospermia (Table 2). This data was evaluated in the context of both the 1999 and 2010 WHO semen analysis norms. Similar results were obtained when examining only the cases with isolated teratozoospermia normal sperm morphology ≤ 4% in terms of fertilization rates and embryo outcomes favoring ICSI over IVF. These findings were noted with normal sperm by both the WHO 1999 criteria (i.e., motility ≥ 50%, concentration ≥ 20 million/ml, volume ≥ 2 ml) and again with 2010 WHO criteria (i.e. forward motility ≥ 32%, concentration ≥ 15 million/ml, volume ≥ 1.5 ml).

When isolating the group with unexplained infertility (N = 29), a higher number of 2PN embryos were seen with ICSI as compared to IVF (3.4 ± 2.3 vs. 2.3 ± 2.2, $p = 0.015$). There was a trend toward a higher mean number of oocytes with fertilization failure (0PN) with IVF as compared to ICSI (1.9 ± 2.5 vs. 0.9 ± 1.1, $p = 0.053$), which was just subsignificant. There were six cases of complete fertilization failure in the IVF group, and none in ICSI which was statistically significant ($p = 0.029$). No statistically significant differences were seen in the number of blastocysts between ICSI and IVF (1.9 ± 1.7 vs. 1.3 ± 1.5, $p = 0.13$) and the number of good-quality blastocysts between the two groups (1.3 ± 1.5 vs. 0.9 ± 1.3, $p = 0.24$) (Table 3).

Pregnancy outcome data are presented in Table 4. It should be noted as discussed elsewhere that significant bias exists in the pregnancy outcome data, in that if there are no embryos from IVF, then mostly there are ICSI embryos that can be transferred. The reverse did not occur. Twenty-five out of 103 patients underwent freeze all for risk of ovarian hyperstimulation syndrome. The remaining 78 patients underwent a fresh embryo transfer, the results of which are

Table 2 Comparison between IVF and ICSI when normal forms ≤ 4% and normal forms > 4%

	Normal forms ≤ 4% (*N* = 61)			Normal forms > 4% (*N* = 42)		
	IVF (*N* = 61)	ICSI (*N* = 61)	*p*	IVF (*N* = 42)	ICSI (*N* = 42)	*p*
Number of MII	5.8 ± 4.6 (363)	5.4 ± 4.0 (348)	0.24	6.1 ± 3.8 (243)	5.4 ± 3.3 (208)	0.03
Number of 2PN	2.4 ± 2.7 (138)	4 ± 3.4 (259)	**< 0.0001**	3.6 ± 3.3 (141)	3.9 ± 2.8 (154)	0.39
Number of 0PN	3.1 ± 3.9 (197)	1.2 ± 1.6 (74)	**< 0.0001**	1.8 ± 2.3 (71)	1.2 ± 1.6 (47)	0.29
Number of day 2 embryos	2.3 ± 2.7 (136)	4.0 ± 3.4 (260)	**< 0.0001**	3.6 ± 3.3 (139)	3.8 ± 2.7 (147)	0.69
Number of day 3 embryos	2.2 ± 2.7 (129)	4 ± 3.4 (258)	**< 0.0001**	3.4 ± 3.2 (131)	3.8 ± 2.8 (147)	0.39
Number of day 5 embryos	1.2 ± 2.0 (69)	2.2 ± 2.6 (146)	**0.001**	2.3 ± 2.6 (88)	2.3 ± 2.4 (91)	0.91
Number of good-quality blastocysts	0.9 ± 1.8 (53)	1.4 ± 1.7 (93)	0.062	1.6 ± 2.1 (62)	1.8 ± 2.1 (70)	0.62
Comparison between IVF and ICSI when normal forms ≤ 4% with normal sperm parameters based on 2010 and 1999 WHO criteria						
	2010 WHO criteria (*N* = 45)			1999 WHO criteria (*N* = 26)		
	IVF (*N* = 45)	ICSI (*N* = 45)	*p*	IVF (*N* = 26)	ICSI (*N* = 26)	*p*
Number of MII	5.5 ± 4.4 (213)	4.9 ± 3.9 (212)	0.12	5.8 ± 5.08 (159)	5.3 ± 4.6 (155)	0.37
Number of 2PN	2.2 ± 2.7 (90)	3.5 ± 3.0 (155)	**0.003**	2.3 ± 3.1 (61)	3.8 ± 3.4 (113)	**0.02**
Number of 0PN	2.8 ± 3.9 (106)	1.1 ± 1.6 (48)	**0.002**	3 ± 4.4 (85)	1.3 ± 1.8 (36)	**0.03**
Number of day 2 embryos	2.2 ± 2.6 (88)	3.6 ± 3.0 (156)	**0.001**	2.3 ± 3.0 (60)	3.8 ± 3.5 (114)	**0.01**
Number of day 3 embryos	2.2 ± 2.7(83)	3.5 ± 3.0 (155)	**0.003**	2.1 ± 3.0 (55)	3.8 ± 3.5 (113)	**0.01**
Number of day 5 embryos	1.1 ± 2.0 (42)	2.0 ± 2.7 (90)	**0.004**	1.3 ± 2.5 (33)	2.3 ± 3.2 (68)	**0.03**
Number of good-quality blastocysts	0.9 ± 1.8 (34)	1.2 ± 1.5 (52)	0.28	1.0 ± 2.3 (26)	1.2 ± 1.5 (36)	0.63

Values are presented as mean ± SD, and absolute numbers are presented in parenthesis. ICSI is statistically favored over IVF in cases with morphology ≤ 4% and normal sperm parameters based on 2010 WHO criteria and 1999 WHO criteria

Table 3 Comparison between IVF and ICSI in unexplained infertility group

	IVF ($N=29$)	ICSI ($N=29$)	p
Number of MII	5 ± 2.3 (145)	4.5 ± 2.2 (131)	0.16
Number of 2PN	2.3 ± 2.2 (67)	3.4 ± 2.3 (99)	**0.015**
Number of 0PN	1.9 ± 2.5 (56)	0.9 ± 1.1 (26)	0.053
Number of day 2 embryos	2.3 ± 2.1 (66)	3.2 ± 1.9 (92)	0.058
Number of day 3 embryos	2.2 ± 1.9 (63)	3.2 ± 2.3 (95)	0.07
Number of day 5 embryos	1.3 ± 1.5 (39)	1.9 ± 1.7 (54)	0.13
Number of good-quality embryos	0.9 ± 1.3 (28)	1.3 ± 1.5 (37)	0.24
Frozen blastocysts	0.8 ± 1.3 (24)	0.9 ± 1.3 (26)	0.78

Values are presented as mean ± SD, and absolute numbers are presented in parenthesis. A statistically significant difference in favor of ICSI was noticed in the fertilization rate

presented in Table 2. Forty-three patients underwent transfer of ICSI embryos only. Twenty-three patients underwent transfer of IVF embryos only. Twelve patients had a transfer of both an IVF and an ICSI embryo and were excluded from the comparison (Table 2). Among ICSI patients, an average of 1.1 ± 0.3 embryos were transferred, while among IVF patients 1.2 ± 0.4 embryos were transferred ($p=0.16$, variances differ, unpaired t test). Among ICSI patients, 14 (33%) had day 3 transfers and 29 had day 5 blastocyst transfers. Among IVF patients 8 (35%) had day 3 embryo transfers and 15 had day 5 blastocyst transfers. No day 2 embryo transfers were performed. There was no statistically significant difference between ICSI and IVF in terms of: pregnancy rates, clinical pregnancy rates, miscarriage rates, or live birth rates, even when stratified for normal sperm morphology groupings. However, clearly ICSI rescued the pregnancy rates in the cycles where IVF fertilization did not occur, which is particularly visible in the group with normal morphology ≤ 4%. In this group, ICSI embryos constituted 74% of embryo transfers when excluding those that had both an ICSI and IVF embryo to transfer.

Discussion

This study evaluates the cases of patients based on strict sperm morphology in whom sibling oocytes were treated with split insemination by conventional IVF and ICSI in their first ART cycle. The oocytes inseminated by ICSI were found to have a significantly higher fertilization rate than those inseminated by conventional IVF when normal sperm morphology ≤ 4%, yielding a significantly higher mean number of embryos, a non-significant trend of higher-quality blastocysts, as well as higher mean number of good-quality embryos available for cryopreservation. Complete fertilization failure was significantly higher following conventional IVF as compared to ICSI in this group, but not in the group with normal strict sperm morphology > 4%.

An additional 48 immature oocytes underwent spontaneous maturation in the IVF group, giving a quantitative advantage to the IVF over ICSI (606 vs. 556 mature oocytes, respectively). ICSI, however, gave a higher fertilization rate despite this quantitative advantage. It is possible that these additional approximately 10% of oocytes that matured in the IVF group may have had a possible maturation defect precluding fertilization. However, it is unlikely this would have led to complete fertilization failure in any patient, nor is it likely that this was the cause of the approximately 50% increase seen in the number of embryos and blastocysts when ICSI was used in the group with normal sperm morphology of ≤ 4%. We also would have expected these oocytes to give a disadvantage to the IVF group with normal sperm morphology > 4%; however, such a difference was not seen.

No statistically significant difference was noticed when examining the rates of implantation, pregnancy, live birth and spontaneous miscarriages at a single transfer. However, a significant bias exists in this data. In 23 out of 24 cases where IVF failed to result in fertilization, ICSI did result in fertilization and in an embryo transfer in these cases. However, had IVF alone been performed given the rate of complete fertilization failure, the pregnancy rates likely would have been statistically lower in this group than in a group

Table 4 Clinical outcomes in patients who had only IVF or ICSI embryos transferred

	All subjects			Normal sperm morphology ≤ 4%			Normal sperm morphology ≥ 5%		
	IVF ($N=23$)	ICSI ($N=43$)	p	IVF ($N=9$)	ICSI ($N=26$)	p	IVF ($N=14$)	ICSI ($N=17$)	p
Pregnancy rate %	61% (14)	42% (18)	0.11	67% (6)	35% (9)	0.09	57% (8)	53% (9)	0.82
Clinical pregnancy rate %	43% (10)	28% (12)	0.20	56% (5)	27% (7)	0.12	36% (5)	29% (5)	0.71
Spontaneous abortion rate %	71% (10/14)	67% (12/18)	0.77	50% (3/6)	56% (5/9)	0.83	88% (7/8)	78% (7/9)	0.60
Live birth rate %	17% (4)	14% (6)	0.71	33% (3)	15% (4)	0.25	7% (1)	12% (2)	0.66

Absolute numbers are expressed in parenthesis

randomized to 100% ICSI, although this cannot be known with certainty. It should also be noted that when considering the cumulative birth rate, ICSI likely would be favored over IVF, as the number of embryos and the quality of frozen embryos with ICSI was higher than in IVF.

These differences in laboratory results between IVF and ICSI were also noticed when examining the cases with isolated teratozoospermia ≤ 4% and normal sperm parameters based on the 1999 WHO sperm criteria as well as on the 2010 WHO criteria. However, no such differences were seen in cases with normal sperm morphology ≥ 5%, which further emphasizes the importance of teratozoospermia as an isolated factor.

However, the group with normal sperm morphology ≥ 5% is small ($N = 42$) and, when performing a power analysis, it seems more than 1000 patients are needed to demonstrate a difference in the number of 2PN embryos given the rates that occurred in this group. Therefore, it is likely that the use of ICSI is not clinically indicated in this group. The results from this study demonstrated a value for ICSI when isolated teratozoospermia were present.

At least one group has suggested that split IVF and ICSI insemination should be performed on sibling oocytes (the IVF–ICSI split procedure) in patients with male subfertility, unexplained infertility and previous unexplained fertilization failure [26]. However, decisions concerning the treatment choice for assisted reproduction (IVF or ICSI) are usually based on the results of previous IVF attempts and on the existence and degree of male fertility factor. Clear-cut rules, however, do not exist for choosing one technique over the other. It is important to note that ICSI has higher costs, procedure times [27], chances of damaging the oocyte [28], and possibly higher rates of fetal anomalies [29]. Based on the results in this study, particularly a higher complete fertilization failure rate in IVF as compared to ICSI in cases with teratozoospermia and in cases with unexplained infertility, it seems reasonable to choose the IVF–ICSI procedure in these subjects.

The possible negative effect of isolated teratozoospermia on IVF is controversial. Several previous studies have shown lower rates of fertilization, implantation, pregnancy, and lower-quality embryos in IVF cycles of teratozoospermic patients [11–13]. However, a study by Keegan et al. found no improvement in IVF outcomes when ICSI was used to treat couples with isolated teratozoospermia. It should be noted that sibling oocytes were not used in that study [15]; however, Fan et al. found similar results using sibling oocytes [16]. Nevertheless, our results of higher fertilization rates in ICSI and teratozoospermia are comparable with many previous studies. Several of these studies included men with other abnormal sperm parameters than morphology [12, 28, 30–33], while others evaluated teratozoospermia as an isolated factor [9, 11–14, 17, 30, 34]. The weaknesses of some of these previous studies are including patients with repeated IVF failures and that IVF and ICSI were performed in different patients and not in sibling oocytes.

In this study, an advantage of ICSI over IVF when normal sperm morphology ≤ 4% was in obtaining a higher number of cleavage stage and day 5 embryos as well as better-quality blastocysts (Table 2). Though most studies have found similar results concerning the higher rates of fertilization in ICSI as compared with IVF, it is not the case with the number and quality of embryos [9, 11–14, 17, 30, 34]. In our study, a higher number of good-quality blastocysts were found in ICSI when the entire group was evaluated; nevertheless, this finding was attributable to the group with normal sperm morphology of ≤ 4%. Pisarska et al. in a prospective study using sibling oocytes and isolated severe teratozoospermia, ICSI resulted in higher fertilization rates than conventional IVF, but without altering embryo quality [13]. However, this study was underpowered (38 patients) which may have contributed to their findings. In another study, despite higher fertilization rates in ICSI than IVF oocytes, ICSI-derived embryo development was compromised compared with IVF [12]. This result is the opposite of what was found in this study. These conflicting findings require further investigation.

Similar to the findings in this study, Oehninger et al. and Fishel et al. found that ICSI produced better-quality embryos, in couples with severe teratozoospermia than with IVF [14, 17]. However, in the Fishel study, these couples had a history of failed fertilization with conventional IVF and may, therefore, represent a different population of patients.

Most studies reported fertilization rates and laboratory results without following the pregnancy outcomes. Mansour et al. [35], found no difference in fertilization and pregnancy rates between patients with ≥ 5% normal sperm morphology using strict criteria and those with < 5% normal sperm morphology when using ICSI for both.

A meta-analysis, which questioned the association between severe teratozoospermia and clinical pregnancy after IVF with or without ICSI, concluded that isolated teratozoospermia was not associated with decreased clinical pregnancy rates with IVF with or without ICSI. However, this meta-analysis found that teratozoospermia has been associated with poor fertilization using conventional IVF, whereas fertilization with ICSI was not affected. One weakness of this meta-analysis was that none of the studies included used sibling oocytes to account for uncontrollable differences. A second weakness was that the cumulative pregnancy rate was not evaluated, which may have differed if IVF affected fertilization outcomes [34].

It should be noted that ultimately more MII oocytes underwent IVF than ICSI. This is because immature oocytes will undergo maturation when not stripped and can be fertilized. This phenomenon has been previously described

[19]. When ICSI is not indicated, this phenomenon results in more embryos and more high-quality embryos than does ICSI with its resultant arrested oocyte development [19]. Just the opposite was noted in this study, with more embryos occurring in the ICSI group, which confirms the value of ICSI in the population with low strict morphology.

It should be acknowledged that published studies on isolated teratozoospermia were based on the old WHO sperm analysis criteria of 1990 and 1999. This study is the first to examine the difference between ICSI and IVF on sibling oocytes in isolated teratozoospermia based on the 2010 WHO criteria.

One question which has arisen is that since normal sperm morphology was measured several months before the IVF procedure and not on the day of insemination, the results may have changed and altered outcomes. This is unlikely to have occurred. Had it been the case, we would have expected the low morphology group to perform similarly to the group with normal morphology > 4% with no difference in IVF and ICSI outcomes (had strict morphology improved). Concurrently, had there been a deterioration in the percent normal forms in the group with > 4% normal morphology, ICSI should have been favored in this group. However, since we found the differences favoring ICSI only in the groups with normal sperm morphology ≤ 4% at baseline testing, we anticipate that the evaluation several months prior to the IVF cycle maintained its clinical relevance.

Another value of our study exists in using sibling oocytes, thereby eliminating any female contribution, or undetected biases to the couple's infertility. The enrollment of the patients was done in a prospective fashion. The database also had a control population with strict morphology > 4% normal forms. This database included both isolated and non-isolated teratozoospermia which permitted the determination of whether the effect was due to morphology or other semen parameters as a confounding effect. Another strength of the study is inclusion of each patient only once for their first IVF cycle, preventing decisions based on previous poor fertilization and effects of repetitive inclusion. No additional cost was accrued by ICSI which was covered by the Quebec government in all cases at the same cost as standard IVF without ICSI.

It should be noted that Table 4 reports a 71% miscarriage rate with IVF and a 67% miscarriage rate with ICSI, with a live birth rate of 17% in IVF and 14% in ICSI. This rate of miscarriage is higher than those reported in the literature as well as higher than those at our clinic. However, the sample size is small ($N = 66$, who did not perform a freeze all cycle). In addition, the average age of the female patients participating in the study was 35 years. Clearly, older age can contribute to higher miscarriage rates and lower live birth rates. It should also be noted that the best responders who would have been expected to have the highest live birth rates underwent freeze all cycles, and were not included in Table 4, likely affecting the rates seen. This pregnancy data however is extremely biased because the ICSI protected the IVF failed fertilization group. The truth is that the pregnancy results are not an outcome of this study, but fertilization is. However, we realized that not including the pregnancy outcomes was an issue for some readers. Hence it was included. However, we do not believe given the bias in the pregnancy data, any conclusion should or can be drawn from them.

Infertile males often exhibit unconventional semen parameters, including DNA fragmentation, chromatin dispersion, and aneuploidy—collectively referred to as sperm genome decay (SGD).

The biological impact of an abnormal sperm chromatin structure depends on the combined effects of the extent of DNA or chromatin damage in the spermatozoa and the capacity of the oocyte to repair that damage [36]. It has been shown that all DNA repair pathways are potentially functional in human oocytes and blastocysts [37]. Even if the fertilizing spermatozoon carries DNA damage in its genome, the oocyte, zygote and blastocyst could potentially repair this damage. It was suggested that the oocyte has the capacity to repair DNA damage of sperm when it is damaged by less than 8%; it also depends, among others, on the DNA damage type, as single-stranded DNA damage is easier to repair than the double-stranded type [38]. The oocytes efficiency of DNA repair decreases with increase in maternal age [39]. Unfortunately, currently available tests cannot provide information concerning the "reparability" of sperm DNA damage. A recent meta-analysis [40] that included 15 prospective studies supports an association between sperm DNA fragmentation and recurrent pregnancy loss. It should be noted that SGD is beyond the scope of this study and was not routinely analyzed in our patients. It is possible that this undetected factor has contributed to the high miscarriage rates seen and should be investigated in future studies.

In conclusion, in couples with severe teratozoospermia, regardless of other sperm parameters, there is an advantage of ICSI over IVF in terms of fertilization rate, quantity and quality of cleavage stage embryos and blastocysts. It is likely as compared to 100% IVF that the pregnancy and cumulative live birth rates are improved by ICSI. However, it should be acknowledged that split insemination may be sufficient to result in similar live birth outcomes in the fresh cycle, although cumulative live birth rates in theory may be compromised. Based on these results, the ICSI procedure seems reasonable as a first-line treatment in patients with normal sperm morphology ≤ 4%, as well as in patients with unexplained infertility.

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ST: manuscript editing, final approval of the version submitted.

WYS: project development, manuscript editing, final approval of the version submitted.

MHD: project development, data management and analysis, manuscript editing, final approval of the version submitted. Dr Dahan acted as the senior author of this study.

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