10.5005/jp-journals-10016-1114

RESEARCH ARTICLE

Influence of Sperm Morphology on the Outcome of Assisted Reproductive Technique—Intracytoplasmic Sperm Injection Cycles: A Retrospective Analysis

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ABSTRACT

Aim: The aim of this study was to evaluate the influence of spermatozoa morphology on ICSI cycle outcome parameters in couples with male factor infertility.

Design: Retrospective study.

Setting: University-level tertiary care center.

Patients and methods: One hundred and forty-eight couples with male factor infertility who had undergone intracytoplasmic sperm injection (ICSI) cycle from 2010 to December 2012 were included in this analysis. The semen samples of the male partners were classified according to the three predictive categories of the Tygerberg strict criteria: excellent prognosis (> 14% morphologically normal spermatozoa), good prognosis (5–14%) and poor prognosis (< 5%).

Main outcome measures: The primary outcome was the embryo quality rate.

Results: Patients in the poor prognosis subgroups exhibited deficits in spermatozoa concentration, motility and total motile fraction. The variations in the outcome parameters of fertilization rate, embryo development rate and embryo quality did not correlate with sperm morphology.

Conclusion: Our study suggests that Kruger's strict morphology criteria of the fresh semen sample is not a good predictor for the ICSI cycle outcome.

Keywords: Embryo quality rate, Intracytoplasmic sperm injection, Sperm morphology.

How to cite this article: Preetha RP, Kamath MS, Aleyamma TK, Muthukumar K. Influence of Sperm Morphology on the Outcome of Assisted Reproductive Technique-Intracytoplasmic Sperm Injection Cycles: A Retrospective Analysis. Int J Infertil Fetal Med 2015;6(3):122-127.

Source of support: Nil

Conflict of interest: None

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Date of received: 27-09-15

Date of acceptance: 10-10-15

Date of publication: December 2015

INTRODUCTION

Semen analysis is routinely performed to evaluate the male partner during an infertility work-up and sperm morphological assessment is considered an important parameter of the investigation. The assessment of sperm morphology based on 'strict morphological criteria[1-3] can be used to discriminate three categories in relation to the predicted outcome of standard assisted reproductive technique (ART) treatment: excellent (> 14% morphologically normal spermatozoa), good (5–14%) and poor prognosis (< 5%).[4] Palermo et al reported that none of the sperm parameters correlated with the outcome of assisted reproductive technique—intracytoplasmic sperm injection (ART-ICSI) cycles.[5] Earlier studies have shown that with ART-ICSI cycles, semen samples with poor Kruger morphology have similar fertilization and pregnancy rates to those with normal morphology.[6-17]

Present literature is conflicting with regards to influence of sperm morphology on ART-ICSI cycles outcomes.

MATERIALS AND METHODS

Records of couples undergoing ART-ICSI cycles in a university level ART center from Jan 2010 to Dec 2012 were collected and analyzed retrospectively. The project was approved by Institutional Review Board. Couples with male factor infertility who had a semen analysis with morphological assessment using Kruger strict criteria before undergoing their intracytoplasmic sperm injection (ICSI) cycle were included in the study.

Semen samples with insufficient spermatozoa for morphologic assessment, surgically retrieved semen sample, female partner with age ≥ 38 years, pelvic pathology like large fibroid, severe endometriosis, hydrosalpinx, etc. were excluded from the study to minimize their contribution as confounding variables.

Spermatozoa Morphology Assessment

A routine semen analysis was done with assessment of concentration, progressive motility and sperm morphology

which was assessed according to the Tygerberg criteria.[1] In brief, a 5 µL droplet of the semen sample was smeared on a prestained-slide (Test Simplet). For each semen sample, at least 200 spermatozoa were examined microscopically at 100× magnification under oil immersion in phase contrast microscope. Ongoing quality control over sperm morphology assessment was done by scoring in agreement with two observers and duplicating each slide. The semen samples were classified according to the three predictive categories of the Tygerberg strict criteria: excellent prognosis (>14% morphologically normal spermatozoa), good prognosis (5–14%) and poor prognosis (<5%).[10]

Stimulation and ART Protocol

Protocols for stimulation included standard long agonist protocol, antagonist protocol, short flare protocol or ultra-long depot protocol depending upon the patient characteristics. Semen samples used for ART-ICSI were prepared by density-gradient method. Oocytes were recovered by transvaginal aspiration of follicles under ultrasound guidance and were cultured in fertilization medium under an oil overlay. Culture dishes were incubated at 37°C with 6% CO_2, 5% O_2, 89% N_2 for 2 to 3 hours. ICSI was performed 3 to 4 hours after oocyte retrieval. Fertilization check was performed 18 to 20 hours after the ICSI procedure. Normal fertilized zygotes were then placed in micro drops containing cleavage medium and cultured for an additional 1/2 days. Embryo transfer was performed on day 2/3 under ultrasound guidance. Day 5 transfer was undertaken if there was > 4 grade—1 embryo present on day 3. A maximum of three embryos were transferred depending upon the different parameters like female partner's age, indication, previous failure, etc. Pregnancy testing was performed 18 days after the oocyte retrieval. Clinical pregnancy was confirmed by the presence of a fetal heart on ultrasound examination at 6 to 8 weeks of pregnancy.

The primary outcome was the embryo quality rate (defined as the number of grades 1, 2 and 3 embryos on day 2 and 3 out of the total number of embryos developed). Grading of embryos was according to criteria laid down by the Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group Embryology in Istanbul consensus workshop on embryo assessment in 2011.[18] Secondary outcome measures were fertilization rate, blastulation rate, implantation rate, clinical pregnancy rate, miscarriage rate and live birth rate.

Statistical Analysis

Differences in outcome measures between groups were compared using the Chi-squared test (for continuous variables) and the Student t-test (for categorical variables) using statistical package for the social sciences (SPSS) 14 software.

RESULTS

A total of 148 ART-ICSI cycles were included in the analysis, of which 89 cycles belonged to group A (poor prognosis) and 59 cycles to group B (good prognosis). There were no patient who belonged to group C (Kruger's criteria >14). The base-line characteristics, such as age, body mass index, type of infertility, type of protocol used for stimulation, were comparable between the two groups (Table 1). Patients in the group A also exhibited abnormality in spermatozoa concentration, motility and total motile fraction (post wash). Mean fresh concentration and motility showed a statistically significant difference when both the groups were compared. Overall, 77% of male partners were diagnosed to

Table 1: Baseline characteristics

		Group A	*Group B*	*p-value*
Age	Male	37.29 (5.4)	37.68 (5.4)	0.7
Mean (*SD)	Female	30.6 (4.3)	31.8 (3.9)	0.1
Infertility	Primary	75 (84)	49 (83)	0.8
n (%)	Secondary	14 (16)	10 (17)	
†BMI	≤ 24	48 (54)	28 (48)	0.44
n (%)	>24	41 (46)	31 (53)	
Type of protocol	Long	45 (51)	32 (54)	0.8
n (%)	Short	5 (6)	4 (7)	
	Antagonist	38 (43)	23 (39)	
Mature oocytes	<4	17 (19)	13 (22)	0.8
obtained n (%)	4–10	54 (61)	33 (60)	
	>10	17 (19)	13 (22)	
Day of embryo transfer n (%)	Day 2	13 (15)	8 (14)	0.2
	Day 3	70 (78)	42 (71)	
	Day 5	6 (7)	9 (15)	

*Standard deviation, †Body mass index

have severe oligoasthenozoospermia, 85% in group A and 64% in group B, the difference being statistically significant (p-0.009). A total of 1058 oocytes were injected, out of which 769 got fertilized (73%). Total number of oocytes obtained was similar in both groups (Table 2). The variations in the outcome parameters of fertilization rate and embryo development rate did not correlate with sperm morphology. Embryo quality represented by grades 1, 2 and 3 also had no significant correlation with the morphology groups. Mean embryos transferred were 2.29. Even though there was no statistically significant difference seen in the day of embryo transfer, group B patients (15%) had higher rate of embryo transfer at blastocyst stage than group A (7%) patients. Likewise, implantation rate, pregnancy rate and clinical pregnancy rates were also similar in both the groups. While the miscarriage rate was higher in group A (13.2%) when compared to group B (3.4%), the difference was not found to be statistically significant. The difference in live birth rate among the two morphological groups was also not statistically significant (Table 3). There was only one patient who belonged to group B who had delivered a baby with major congenital anomaly.

DISCUSSION

In our study, we analyzed and compared the embryonic development rate, pregnancy outcomes and live birth rate between two groups of couples undergoing ART-ICSI cycles for male factor infertility; the groups divided on the basis of difference in proportion of morphologically normal sperm. We found no significant relation of sperm morphology with ICSI outcome parameters, such as fertilization rate, embryo development rate, embryo quality rate, pregnancy rate, miscarriage rate and live birth rate. Our findings are in agreement with previous studies which also did not find any correlation of sperm morphology with ART-ICSI outcomes.[8,12,13,19]

Accurate sperm morphology evaluation is crucial to the routine examination of semen because the percentage of morphologically normal sperm represents an important predictor of male fertilizing potential.[20,21] The reference staining method for sperm morphology assessment is the Papanicolaou technique,[22] and it has been used in several studies as the reference to validate other staining methods, such as Shorr and Diff-Quik (DQ). In this study, we used the commercially available Testsimplets (TS), as a valid alternative for the evaluation of sperm morphology,[23,24] thus, avoiding the use of chemical reagents and in particular of volatile compounds that could contaminate the environment in which embryos are cultured. In a recent study,[25] the TS method gave a lower number of normal forms compared with the results obtained by the DQ technique. Ragni et al[26] also reported that the TS technique detects a higher number of sperm anomalies than Papanicolaou, modified Giemsa, Hemaquick, and hematoxylin-eosin staining techniques. This might be one

Table 2: Male factor category and semen parameters

		Group A	*Group B*	*p-value*
Male factor	Severe *OATS	76 (85)	38 (64)	0.009
n (%)	Mild OATS	9 (10)	17 (29)	
	Isolated teratozoospermia	4 (5)	4 (7)	
Semen parameters	Concentration	11.7 ± 15.4	24.7 ± 29.8	0.003
Mean ± SD	Motility	20.5 ± 15.1	32.9 ± 18.1	0.000
	Volume	2.6 ± 1.42	2.7 ± 1.48	0.48
	†FSH value	8.1 ± 5.2	10.6 ± 7.2	0.176
	Total Motile Fraction	3.5 ± 6.1	10.5 ± 16.4	0.004

*Oligoasthenoteratozoospermia †Follicle Stimulating Hormone

Table 3: Outcome parameters

		Group A	*Group B*	*p-value*
Fertilization rate n (%)		466/651 (72)	303/407 (74)	0.39
Embryo development rate n (%)		449/466 (96)	299/303 (99)	0.08
Embryo quality rate n (%)	Grade 1	173/449 (39)	131/299 (44)	0.14
	Grade 2	161/449 (36)	93/299 (31)	0.17
	Grade 3	113/449 (25)	73/299 (24)	0.7
Blastulation rate (Mean ± SD)		42.03 ± 20.9	50.30 ± 23.57	0.30
Implantation rate n (%)		42/188 (22)	29/134 (22)	0.98
Pregnancy rate n (%)		42 (47)	31 (53)	0.52
Biochemical pregnancy rate n (%)		3/42 (7)	3/31 (9.6)	0.92
Clinical pregnancy rate n (%)		36 (40)	28 (47)	0.39
Miscarriage rate n (%)		5 (13.2)	1 (3.4)	0.34
Live birth rate n (%)		33/89 (37)	26/59 (44)	0.26

of the reasons for the higher percentage of patients with morphology <5% (poor prognosis group) in our study.

Oligozoospermic semen samples may exhibit abnormalities of sperm morphology.[27] In the present study, the spermatozoa concentration, motility and total motile fraction varied considerably within the two morphology categories.

Failed fertilization is a general event observed in approximately 30% of oocytes after ICSI. Although more than 80% of these oocytes contain spermatozoon, failed fertilization in these oocytes has been attributed to inability of these oocytes to initiate the biochemical processes necessary for oocyte activation.[28] Failed fertilization due to sperm factors has been related to sperm morphology, sperm nuclear morphology, acrosomal defects, and sperm chromatin status.[1,29] But according to Svalander et al,[10] sperm morphology may not be a critical factor for fertilization using ICSI because many natural processes, such as the penetration of the zona pellucida are bypassed. In the present study, no correlation was observed between sperm morphology and fertilization rate after ICSI.

We observed that the embryo quality in both the groups were similar, indicating that the morphological abnormality of spermatozoa may not be linked to genetic abnormality of the male gametes and its inability to penetrate the egg as suggested by earlier studies.[30,31]

According to Kihaile et al, development was compromised in the ICSI group, with fewer embryos progressing to the blastocyst stage when patients with severe teratozoospermia were compared for IVF and ICSI.[32] But according to Van Landuyt et al, sibling oocytes from ICSI *vs* conventional IVF showed similar rates of embryonic development and blastocyst formation.[33] French et al could not find a negative effect of ICSI technique on blastocyst formation in his study with 1074 ICSI cycles and also did not observe a correlation between severe teratozoopsermia and poorer blastocyst quality. Furthermore, a significantly greater percentage of high-quality blastocysts was obtained in the most severely teratozoospermic subgroup, with 0% normal forms.[34] Present study also could not find a correlation between sperm morphology and blastulation rate, thus suggesting that Kruger's strict morphology may not be useful in predicting either the rate of blastocyst development or the morphologic characteristics of the resulting blastocysts in ICSI cycles.

Pregnancy rates were uniformly similar in both the groups, independent of the extent of the morphological impairment of spermatozoa. It has been reported that in couples with severe teratozoospermia, the spontaneous term pregnancy rate is very low, whereas the miscarriage rate is higher than in patients with normal sperm morphology.[35] In the present study, although high fertilization and cleavage rates were achieved, a high incidence of early pregnancy loss was seen in the group with poor prognosis compared to the group with good prognosis, but the difference was not statistically significant.

The potential relationship between sperm shape or morphology and chromosomal integrity has become very relevant since the introduction of ICSI.[34] Data from several studies suggest that normal morphology is not a valid indicator for selection of sperm with haploid nuclei.[30,31,36] Celik-Ozenci et al,[37] using fluorescent *in situ* hybridization, found that 10% of sperm with disomic nuclei were categorized as normal by strict morphology.

There is continued concern that the use of the ICSI technique eliminates any opportunity for 'natural selection' of the sperm that will ultimately fertilize the oocyte. Although the risk of birth defects in children conceived through ART is increased over that of natural conception, the fear that ICSI will result in higher rates of major birth defects compared with conventional IVF has not been substantiated by current data. A recent meta-analysis showed a 30 to 40% increased risk of major birth defects with assisted reproduction (either IVF or ICSI) compared with natural conception.[38] No difference was seen in the risk of birth defects when comparing traditional IVF and ICSI.[39] A meta-analysis of four studies with a total of 5,395 children born after ICSI confirms a lack of statistically significant increase in birth defects with ICSI compared with conventional IVF.[40] In the present study, there was only one baby born with major congenital anomaly conceived through ICSI, who belongs to the group B (good prognosis). However, the due to low numbers in the study, it is difficult to make any firm conclusion with regards to congenital anomalies.

This study did not contain a single patient with total teratozoospermia. So as long as there is a morphologically normal spermatozoon available for injection, it seems that the outcome of ICSI is not related to the incidence of morphologically abnormal spermatozoa in the sample.[10] The most likely explanation for this is that during ICSI the embryologist can microscopically select individual sperm that appear morphologically 'normal', from even the most impaired specimens. A possible additional explanation is that as a consequence of the ICSI technique the morphologically abnormal spermatozoon, which would otherwise fail to penetrate the oocyte, is deposited directly into the cytoplasm. Thus, fertilization occurs with sperm that may not be representative of the sperm population within the entire sample, making the initial semen morphology assessment irrelevant. Thus, the only ultimate criterion for successful ICSI is the presence of at least one living spermatozoon per oocyte in the semen preparation used for microinjection.[8]

The main limitation of this study is its retrospective nature and a prospective randomized study can bring more light to the present knowledge on this aspect and a larger sample size is needed to draw a proper conclusion. The use of prestained slides (Testsimplet) for assessing morphology is another drawback of this study.

Studies assessing sperm morphology of the inseminated spermatozoa during ICSI using special imaging systems suggest that sperm morphology shows a significant and high correlation with fertilization and pregnancy rate.[41,42] This concept has been taken to the subcellular level in new techniques, such as intracytoplasmic morphologically selected sperm injection and motile sperm organellar morphology examination (MSOME). In these techniques, investigators select sperm for ICSI using a higher magnification and allow more critical assessment of sperm morphology. Sperm nucleus morphology by the MSOME method has positively correlated with fertilization and pregnancy rates.[34] Larger studies using this technology may provide stronger correlation of sperm morphology with ART ICSI outcomes.

CONCLUSION

Our study finding suggests that the Kruger's strict morphology criterion for fresh semen sample does not correlate with ART-ICSI outcomes in male factor infertility. Microscopic examination and selection of spermatozoa during ICSI can yielded the similar results in good and poor morphology groups. Kruger's strict criteria of fresh semen sample can be applied for the treatment decision rather than predictor of ICSI cycle outcome. Further larger prospective trials evaluating the influence of sperm morphology after processing with density gradient centrifugation on ICSI cycle may help prognosticate ART cycles outcomes.

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