Original Article

Influence of sperm morphology in ICSI cycle outcomes: a retrospective study

Sazda Khatoon[1], Sonia Malik[2], Ved Prakash[2], Aneesha Grover[2]

[1]Sai College of Medical Science and Technology, Kanpur, Uttar Pradesh, India
[2]Southend IVF & Fertility Center, 2 Palam Marg Vasant Vihar, New Delhi, India

Abstract

Aim: To determine whether sperm morphology has any influence on the assisted reproductive technique (ART) outcome in *in vitro* fertilization (IVF)-intracytoplasmic sperm injection (ICSI) cycles. **Objectives:** To assess whether sperm morphology affects IVF-ICSI outcome. **Method:** This study was a retrospective analysis of data from 1000 couples who had undergone ART cycle at Southend Fertility & IVF, Delhi between January 2016 and January 2021. The recruited patients were divided into two groups: Group A: Group A was the study group, and included 600 patients with an abnormal sperm morphology (morphology of <4%); Group B: Group B was the control group, and included 400 patients with a normal sperm morphology (morphology of >4%). The two groups were compared in terms of fertilization rate, embryo development rate, grade A embryo development rate, grade B embryo development rate, grade C embryo development rate, cleavage rate, embryo discarded or damage rate, and pregnancy rate. **Results:** Of the total 1000 ART-ICSI cycles analyzed the baseline characteristics, such as age, type of infertility, and semen parameters such as volume, count, motility, debris, agglutinations, grade A motility, grade B motility, total progressive (A+B) motility, morphology assessments, and type of protocol used for stimulation were comparable between the two groups. In the 1000 patients analyzed, a total of 6871 oocytes were injected at ICSI, of which 4049 oocytes belonged to group A and 2822 oocytes belonged to group B of the 6871 oocytes injected, 6275 got fertilized, giving a fertilization rate of 91.3%. On comparing the fertilization rate of the two groups, a fertilization rate of 90.1% (3650/4049) was observed in group A and 93.01% (2625/2822) was observed in group B, which was statistically different ($P < 0.001$). Of the various outcome parameters, a statistically significant difference was reported in the fertilization rate, cleavage rate, embryo development rate, grade A embryo development rate, and grade C embryo development rate in the two groups. No statistically significant difference was observed in the pregnancy rate. It was also observed that once fertilization occurred, embryo quality was good for all types of abnormal spermatozoa (grade A, good- to excellent-quality embryos), except for spermatozoa with broken necks, with which only good to excellent quality, or poor quality grade B and group C embryos were obtained. **Conclusion:** Sperm morphology influences the fertilization rate, cleavage rate, and the embryo development rate but there is no significant influence of sperm morphology on pregnancy rate.

Keywords: Assisted reproductive technology, embryo morphology, intracytoplasmic sperm injection, male infertility, oocyte fertilization, sperm morphology

Address for correspondence: Sazda Khatoon, lauwan, Hardiya,via; Barhariya, Siwan, Bihar,.Pin Code 841232, India.
E-mail: sazda.khatoon20@gmail.com
Submission: 19–05–2021, **Revised:** 28–09–2021, **Accepted:** 25–10–2021, **Published:** 29–June–2022

Access this article online	
Quick Response Code:	**Website:** www.fertilityscienceresearch.org
	DOI: 10.4103/fsr.fsr_17_21

How to cite this article: Khatoon S, Malik S, Prakash V, Grover A. Influence of sperm morphology in ICSI cycle outcomes: <?3B2 show $6#?a retrospective study. Fertil Sci Res 2022;9:23-8.

INTRODUCTION

Assisted reproductive techniques (ARTs) have been used with increased frequency since the birth of first *in vitro* fertilization (IVF) baby in 1978, and are the treatment of choice in indicated cases of male and female infertility.[1] Infertility is defined as the failure to accomplish a clinical pregnancy after 1 year or more of regular intercourse as per WHO. Approximately 10% to 15% couples all over the world suffer from infertility, of which 25% to 30% are due to male factors, 20% to 35% are due to female factors, and 20% are unexplained.

Intracytoplasmic sperm injection (ICSI) is an IVF procedure in which a single sperm cell is injected directly into the cytoplasm of an egg to form an embryo which is then transferred into the maternal uterus for obtaining a pregnancy.

The ICSI was first introduced as an add-on IVF procedure in 1992, almost 22 years after the birth of the first IVF baby. Previously ICSI was used for severe male factor infertility and those with unexplained infertility but now has been widely accepted for several other indications such as previous IVF failure.

The ICSI involves sperm selection of live sperms based on their morphologic features observed under an inverted microscope, at an optical magnification of 400×. At this magnification, some of the morphologic anomalies can be detected such as number of tails or other tail and neck abnormalities, or the presence of cytoplasmic droplet. The advantage of this method of sperm selection is that no staining of the sample is involved and thus viability is not affected. However, only major morphologic abnormalities can be detected by this method.

Semen analysis is commonly used in the evaluation of the male partner among infertile couples.[2] Of the various semen analysis parameters, strict Sperm morphology assessment as described by Kruger has been proposed to be one of the most informative in differentiating between fertile and infertile men. It has been considered a biomarker of sperm fertilizing capacity, ICSI is an IVF procedure in which a single morphologically selected sperm cell is injected directly into the cytoplasm of an egg to form an embryo.

Severe abnormalities in sperm morphology, as in the case of teratozoospermia, are associated with poor fertilization during IVF cycles. In such clinical scenarios, ICSI results in higher fertilization rates than conventional IVF, without affecting embryo quality.[3]

The present study was conducted to evaluate whether the sperm morphology has any influence on the ART outcome in ICSI cycles.

MATERIALS AND METHODS

All patients satisfying the inclusion and exclusion criteria were recruited into the study.

Data from 1000 ART cycles of couples who had undegone IVF ICSI cycle at Southend Fertility and IVF between January 2016 and January 2021 were analyzed in this retrospective study.

Data included information on the process of IVF-ICSI such as controlled ovarian stimulation, cycle monitoring, oocyte recovery, insemination or injection of oocytes, fertilization check, and culture of embryos to cleavage or blastocyst stage and finally embryo transfer.

At the time of oocyte retrieval, semen was analyzed for count, motility, and morphology. It was then prepared for use in ICSI using the double density gradient method of sperm separation.

A routine semen analysis was performed according to World Health Organization guidelines (WHO, 2010) using a Makler chamber.

Slides were prepared for sperm morphology assessment using the Diff-Quik method and then assessed for morphology. Sperm morphology grading was carried out according to the Tygerberg criteria.[1,9]

A sperm was considered morphologically normal if the head was normal (normal shape, normal size, having an acrosome, and lacking midpiece or tail defects). Assessment was made by using strict Kruger criteria, to the extent possible given the limited magnification.

The main defects recorded in morphologically abnormal sperms were elongated or tapered head (at least twice smaller in width compared to normal), amorphous head, broken neck (length axis of the head deviated at least 30° from the midpiece axis owing to a breakpoint between the head and midpiece), and presence of a cytoplasmic droplet (regardless of the size of the droplet relative to the head size).

After the oocyte retrieval, the oocytes were incubated for 2 hours and then denuded to remove the surrounding

cumulus in a hyaluronidase-containing medium and then rinsed in HEPES-buffered IVF medium. Five droplets of culture medium, each 5 µL, were placed around one droplet in the center of a plastic culture dish. These were covered immediately with sterile, equilibrated paraffin oil. The center droplet was subsequently replaced by a droplet of polyvinyl pyrrolidone (PVP) solution. Fresh semen sample was taken and prepared. A 1 µL aliquot of the sperm suspension was added to the middle of the PVP droplet. Only mature oocytes having a polar body in the perivitelline space were placed in the media droplets. Sperm morphology was evaluated under the inverted microscope. Selected spermatozoon was immobilized and aspirated into the ICSI pipette and oocyte was inseminated. After injection, the oocytes were transferred to culture dishes containing cleavage medium. Oocytes were then examined 16 to 18 hours after ICSI to assess for fertilization, that is, the presence of two distinct pronuclei and two clear polar bodies. Serial evaluation of embryo morphology was performed as per Istanbul 2010 consensus.[4] The highest morphologically scored embryos were selected for transfer to the patient on the day 2 or 3 or 5. Extra embryos, if available, were cryopreserved by vitrification using Kitazato medium. Pregnancy was detected by measuring serum beta human chorionic gonadotropin levels.

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 version 20 (IBM).

Ethical clearance

The Independent Ethics Committee, Indian Fertility Society Flat No. 302, 3rd Floor, Kailash Building, Kasturba Gandhi Marg, C.P, New Delhi-110001 is registered (Registration ECR/222/indt/DL/2015/RR-18) with Drug Controller General of India, Directorate General of Health Services, New Delhi as per the Rule 122D of the Drugs and Cosmetics Rules 1945.

RESULTS

A total of 1000 ART-ICSI cycles were included in the analysis, of which 600 cycles belonged to group A (poor prognosis cases) and 400 cycles to group B (good prognosis control). The baseline characteristics, such as age, type of infertility, and semen parameters such as volume, count, motility, debris, agglutinations, grade A motility, grade B motility, total progressive (A+B) motility, morphology assessments, and type of protocol used for stimulation, were comparable between the two groups.

Table 1 compares the semen parameters between the two groups. It shows statistically significant difference in motility and morphology in the two groups ($P = 0.001$). Table 2 compares the outcome parameters between the two groups.

Table 1: Male factor category and semen parameters

(Mean ± SD)	Group A	Group B	*P*-value
Male age	36.4 (5.4)	36.9 (5.0)	0.657
Volume	2.1 ± 1.7	2.1 ± 0.9	0.976
Concentration	26.5 ± 22.9	54.0 ± 24.6	<0.001
Motility	41.0 ± 14.9	54.0 ± 24.6	<0.001
Grade A	5.5 ± 4.8	9.3 ± 5.4	<0.001
Grade B	15.3±8.1	20.7±6.9	<0.001
(A+B) progression	20.8±11.7	30.1±11.3	<0.001
Normal morphology	2.4±1.2	4.4±3.1	<0.001
Head defects	61.5±28.8	65.1±7.3	0.004
Neck, midpiece, and tail defects	19.0 ± 10	65.1 ± 7.3	<0.001

Values with the plus/minus sign are the mean and standard deviation (SD).

Table 2: Outcome parameters between the two groups

Outcomes parameters	Group A (case)	Group B (control)	*P*-value
Fertilization rate, *n* (%)	3650/4049 (90.1)	2625/2822 (93.01)	<0.001
Embryo development rate, *n* (%)	3328/3650 (91)	2345/2625(89)	0.014
Embryo quality, grade A (%)	1867/3650 (51)	1278/2625 (48)	0.054
Embryo quality rate, grade B (%)	1071/3650 (30)	721/2625 (28)	0.105
Embryo quality rate, grade C (%)	401/3650 (11)	364/2625 (13)	0.001
Cleavage rate (%)	3328/3650 (91)	2345/2625 (89)	0.014
ICSI damage rate (%)	322/4049 (07)	286/2822 (10)	0.002
Fresh transfer pregnancy rate (%)	131/330(40)	81/200 (41)	0.855
Frozen transfer pregnancy rate (%)	72/43 (60)	28/45 (62)	0.788

Table 3: Baseline female parameters

Female parameters	Group A (case)	Group B (control)	*P*-value
Female age (mean ± SD)	34.5 (5.0)	34.9 (5.0)	0.657
Primary infertility (%)	270 (45)	170 (43)	0.435
Secondary infertility (%)	20 (4)	10 (3)	0.449
Advanced maternal age (%)	107 (18)	101 (25)	0.005
Decrease ovarian reserve (%)	36 (6)	31 (8)	0.278
Female thyroid factors (%)	20 (4)	16 (4)	0.579
PCOD (%)	55 (10)	35 (9)	0.822

Pregnancy outcome is calculated as per embryo transfer per cycle. PCOD, polycystic ovarian disease; SD, standard deviation.

Table 3 compares the baseline characters in the female partner in the two groups. Among all the baseline characters, significant difference was reported in the age between the two groups ($P = 0.005$).

In the 1000 patients analyzed, a total of 6871 oocytes were injected at ICSI, of which 4049 oocytes belonged to group A and 2822 oocytes belonged to group B. Of the 6871 oocytes injected, 6275 got fertilized, giving a fertilization rate of 91.3%. On comparing the fertilization rate of the two groups, a fertilization rate of 90.1% (3650/4049) was reported in group A and 93.01% (2625/2822) was reported in group B, which was statistically different ($P < 0.001$).

The cleavage rates in groups A and B, respectively, were 91% and 89%. In groups A and B, grade A embryo development rates were 51% and 48%, grade B embryo development rates were 30% and 28%, and grade C embryo development rates were 11% and 13%, respectively.

Of the secondary outcome, in fresh embryo transfer cycles, pregnancy rates of 60% in group A and 62% in group B were observed, and a pregnancy rates of 40% in group A and 41% in group B were obtained in frozen embryo transfer cycles. However, the pregnancy rates in the two groups were not found to be statistically significant in both fresh and frozen embryo transfer cycles.

It was also observed that injection of morphologically abnormal spermatozoa did not influence the ICSI survival rate, which was similar for the different types of morphologically abnormal spermatozoa and for morphologically normal spermatozoa (overall, 93% of injected oocytes). Once fertilization occurred, embryo quality was good for all types of abnormal spermatozoa (grade A, good- to excellent-quality embryos), except for spermatozoa with broken necks, with which only good to excellent quality, or poor quality grade B and group C embryos were obtained.

DISCUSSION

In the 1000 ICSI cycles analyzed, we studied the effect of morphologically normal sperm or abnormal sperm cells on ART cycle outcomes. Selection of the "most normal-looking" spermatozoon as observed under the inverted microscope for microinjection is a very important selection step within the procedure. More normal-looking spermatozoa are injected than expected from the Kruger morphology assessment on the semen sample. This study compared ICSI outcomes in couples with normal morphology infertility and male infertility with teratozoospermia to determine if having sperm morphology of less than 4% or greater than 4% were related to the ICSI outcomes.

In the present study, the spermatozoa concentration, total motility, progressive motility(A+B), and other morphology parameters within the two groups were also compared and a statistically significant difference was observed between the two groups in terms of concentration, motility, and morphology parameters, whereas no difference was observed in sperm volume.

Among the primary outcome measures, a statistically significant difference was reported in the fertilization rate, cleavage rate, and embryo development rate between the two groups. However, there was no difference in the pregnancy rates between the two groups. We found in this retrospective study that there is no such difference in fresh embryo transfer pregnancy rates in group A (40%) and group B (41%). On the other hand, in frozen embryo transfer, pregnancy rates in groups A and B were 60% and 62%, respectively, but statically not significant. However, we also observed that frozen embryo transfer pregnancy rate was better than fresh embryo transfer pregnancy rate and this indicates that though morphology affects the embryo development, it does not have any adverse implication on the ART outcome.

As per literature, fertilization failure may be due to sperm factors which have been related to sperm morphology,

sperm nuclear morphology, acrosomal defects, and sperm chromatin status. However, sperm morphology may not be a critical factor for fertilization using ICSI because other processes such as failure of oocyte activation may be involved in this adverse result also.

As given in literature, in our study also we found a higher pregnancy rate after frozen embryo transfer when compared with a fresh embryo transfer. However, the pregnancy rates in the two study groups were not significantly different in either fresh or frozen embryo transfer cycles. This suggests that the morphology of sperms is not related to the success rates of ICSI.

Our results were comparable to the observations of Preetha *et al*.[5] They conducted a retrospective study to understand the influence of spermatozoa morphology on ICSI cycle outcome parameters in couples with male factor infertility, and 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. Various other studies are also in agreement with our observations that there is no correlation of sperm morphology with ART-ICSI outcomes.[6-9] This also suggests that the Kruger strict morphology criterion for fresh semen sample does not correlate with ART-ICSI outcomes, and the microscopic examination and selection of spermatozoa under the inverted microscope during ICSI can yield similar results in good and poor morphology groups.

In another retrospective study, Palermo *et al*.[10] evaluated the assessment of sperm morphology based on "strict morphologic criteria"[11-13] and discriminated three categories in relation to the predicted outcome of standard ART treatment: excellent (>14% morphologically normal spermatozoa), good (5–14%), and poor prognosis (<5%).[7] They reported that none of the sperm parameters correlated with the outcome of ART. This further suggests that sperm morphology does not affect ART outcome.

In ART-ICSI cycles, earlier studies have shown that semen samples with poor Kruger morphology have similar fertilization and pregnancy rates to those with normal morphology.[14] Individual sperm morphology assessed at the moment of ICSI correlated well with fertilization outcome, but did not affect embryo development.[15] The implantation rate was lower when only embryos resulting from injection of an abnormal spermatozoon were available. Literature is contradictory with respect to strict sperm morphology and its effect on ICSI cycle implantation rate.

This was similar to our findings that there was a significant difference in the fertilization rate among the two study groups. However, in our study, no difference was found in the implantation rate between the two groups.

Overall, the findings of the other retrospective studies conducted by Kihaile *et al*. are consistent with the studies by McKenzie *et al*.[16] and French *et al*., which show equivalent outcomes of ICSI cycles for strict sperm morphology of 0% versus morphology of ≥1%. The ability of ICSI to achieve normal fertilization independent of sperm morphology can be explained by the presence of sperm-borne oocyte activating factor.[17,18]

According to another retrospective study conducted by Svalander *et al*.[19] 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 and no correlation was observed between sperm morphology and fertilization rate after ICSI.

However, it is difficult to correlate ICSI to morphology of spermatozoa. ICSI involves the selection of one sperm and injected into cytoplasm of oocytes; all the sperm abnormalities are there by bypassed by using ICSI procedure. In our study also, we did not find any effect of sperm morphology on ICSI cycle outcomes.

This study has certain limitations, including the retrospective nature of the analysis. Also, there may be some female factors which were not diagnosed in the two groups. Moreover, couples who were diagnosed with infertility had more than one factor involved. Apart from the male factor, the female may also be diagnosed with PCOS, advanced maternal age (AMA), poor ovarian reserve (POR), decreased ovarian reserve (DOR), thyroid, endometriosis, tubal factors, and many unknown factors which also affect ART outcomes. Patients' data were collected with either having normal morphology or abnormal morphology criteria, according to Kruger criteria. In this study, most important criterion is to see male sperm morphology effects on the ICSI outcomes. The low magnification and low resolution of the sperm morphology assessment on motile spermatozoa before ICSI is a limitation of our 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. Newer techniques for sperm selection such as intracytoplasmic morphologically selected sperm injection (IMSI) and motile sperm organellar morphology examination (MSOME) have been introduced. This concept has been taken to the subcellular level in new techniques, such as IMSI and MSOME. Larger studies using this technology may provide stronger correlation of sperm morphology with ART-ICSI outcomes.

CONCLUSION

Our study finding concluded that the Kruger strict morphology criterion for sperm morphology does not correlate with ART-ICSI outcomes in male factor infertility. The limitation of this study is its retrospective nature and a prospective randomized study can bring more light to the present knowledge on this aspect. A larger sample size and having genetic information are needed to draw a proper conclusion. Further, larger prospective trials evaluating the influence of sperm morphology after processing with density gradient centrifugation on ICSI cycle may help prognosticate ART cycle outcomes.

Acknowledgments

The authors are grateful to members of the Southened Fertility & IVF center for their assistance in data collection for research purpose.

In particular, the authors acknowledge the senior embryologist Mr Deepu Gupta, Junior embryologist Ms Jyoti Pandey, and other staff members for their assistance.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Kruger TF, Menkveld R, Stander FS, *et al*. Sperm morphologic features as a prognostic factor in in vitro fertilization. Fertil Steril 1986;46:1118-23.
2. Duran EH, Gurgan T, Gunalp S, Enginsu ME, Yarali H, Ayhan AA. A logistic regression model including DNA status and morphology of spermatozoa for prediction of fertilization in vitro. Hum Reprod 1998;13:1235-9.
3. Pisarska MD, Casson PR, Cisneros PL, *et al*. Fertilization after standard in vitro fertilization versus intracytoplasmic sperm injection in subfertile males using sibling oocytes. Fertil Steril 1999;71:627-32.
4. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group Embryology. Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Reprod Bio Medicine Online 2011;22:632-46
5. Preetha PR, Kamath MS, Aleyamma TK, Muthukumar K. Int J Infert Fetal Med 2015;6:122-7.
6. Kupker W, Schulze W, Diedrich K. Ultrastructure of gametes and intracytoplasmic sperm injection: the significance of sperm morphology. Hum Reprod 1998;13(Suppl 1):99-106.
7. Svalander P, Forsberg AS, Jakobsson AH, Wikland M. Factors of importance for the establishment of a successful program of intracytoplasmic sperm injection treatment for male infertility. Fertil Steril 1995;63:828-37.
8. Nagy ZP, Verheyen G, Tournaye H, Van Steirteghem AC. Special applications of intracytoplasmic sperm injection: the influence of sperm count, motility, morphology, source and sperm antibody on the outcome of ICSI. Hum Reprod 1998;13(Suppl 1):143-54.
9. McKenzie LJ, Kovanci E, Amato P, Cisneros P, Lamb D, Carson SA. Pregnancy outcome of in vitro fertilization/intracytoplasmicsperm injection with profound teratospermia. Fertil Steril 2004;82:847-9.
10. Palermo G, Joris H, Derde MP, Camus M, Devroey P, Van Steirteghem A. Sperm characteristics and outcome of human assisted fertilization by subzonal insemination and intracytoplasmic injection. Fertil Steril 1993;59:826-35.
11. Kruger TF, Menkveld R, Stander FS, *et al*. Sperm morphologic features as a prognostic factor in in vitro fertilization. Fertil Steril 1986;46:1118-23.
12. Van Waart J, Kruger TF, Lombard CJ, Ombelet W. Predictive value of normal sperm morphology in intrauterine insemination (IUI): a structured literature review. Hum Reprod Update 2001;7:495-500.
13. Duran EH, Gurgan T, Gunalp S, Enginsu ME, Yarali H, Ayhan AA. A logistic regression model including DNA status and morphology of spermatozoa for prediction of fertilization in vitro. Hum Reprod 1998;13:1235-9.
14. Mansour RT, Aboulghar MA, Serour GI, Amin YM, Ramzi AM. The effect of sperm parameters on the outcome of intracytoplasmicsperm injection. Fertil Steril 1995;64:982-6.
15. Gardner DK, Schoolcraft WB, Wagley L, Schlenker T, Stevens J, Hesla J. Prospective randomized trial of blastocyst culture and transfer in vitro fertilization. Hum Reprod 1998;13:3434-40.
16. McKenzie LJ, Kovanci E, Amato P, Cisneros P, Lamb D, Carson SA. Pregnancy outcome of in vitro fertilization/intracytoplasmic sperm injection with profound teratospermia. Fertil Steril 2004;82:847-49.
17. Neri QV, Lee B, Rosenwaks Z, Machaca K, Palermo GD. Understanding fertilization through intracytoplasmic sperm injection (ICSI). Cell Calcium 2014;55;24-37.
18. Kashir J, Heindryckx B, Jones C, De Sutter P, Parrington J, Coward K. Oocyte activation, phospholipase C zeta and human infertility. Hum Reprod Update 2010;16:690-703.
19. Svalander P, Jakobsson AH, Forsberg AS, Bengtsson AC, Wikland M. The outcome of intracytoplasmic sperm injection is unrelated to 'strict criteria' sperm morphology. Hum Reprod 1996;11:1019-22.