Sperm retrieval and intracytoplasmic sperm injection outcomes with testicular sperm aspiration in men with severe oligozoospermia and cryptozoospermia

Mohannad Alharbi, MD[1,2]; Ahmad Almarzouq, MD[1]; Armand Zini, MD[1,3]

[1]Division of Urology, Department of Surgery, McGill University, Montreal, QC, Canada; [2]Department of Surgery, College of Medicine, Qassim University, Qassim, Saudi Arabia; [3]OVO Fertility Clinic, Montreal, QC, Canada

Cite as: Alharbi M, Almarzouq A, Zini A. Sperm retrieval and intracytoplasmic sperm injection outcomes with testicular sperm aspiration in men with severe oligozoospermia and cryptozoospermia. *Can Urol Assoc J* 2021;15(5):E272-5. http://dx.doi.org/10.5489/cuaj.6798

Published online October 27, 2020

Abstract

Introduction: Several studies addressed the role of testicular sperm aspiration with intracytoplasmic sperm injection (ICSI) in azoospermic men, but few have included non-azoospermic men. The aim of this study was to evaluate testicular sperm aspiration (TESA) sperm retrieval rates and ICSI outcomes in men with severe oligozoospermia.

Methods: Data were collected retrospectively from 88 consecutive, non-azoospermic, infertile men with idiopathic severe oligozoospermia who underwent TESA between January 2011 and January 2018. Patients were categorized into four groups according to sperm concentration: <5 and >1 million/ml (group 1), <1 and > 0.1 million/ml (group 2), <0.1 million/ml (group 3), and cryptozoospermia (group 4).

Results: Mean male age was 37±7 years and the mean female age was 33±4 years. Sperm was recovered successfully in 90% (79/88) of the men overall and in 100% (30/30) of the men in group 1, 97% (29/30) of the men in group 2, 88% (15/17) of the men in group 3, and 45% (5/11) of the men in group 4. Most (65%, 57/88) of the couples had an embryo transfer (ET). The overall clinical pregnancy rate per ET was 46% (26/57). The clinical pregnancy rates (per ET) were 43% (9/21) in group 1, 65% (13/20) in group 2, 36% (4/11) in group 3, and 0% (0/5) in group 4.

Conclusions: Our data indicate TESA allows for high sperm retrieval rates and acceptable ICSI pregnancy rates in men with severe oligozoospermia. However, in our experience, TESA sperm retrieval rates and ICSI outcomes are poor in cryptozoospermic men.

Introduction

Several studies have evaluated the role of testicular sperm aspiration (TESA) with intracytoplasmic sperm injection (ICSI) in azoospermic men, but few have examined TESA in non-azoospermic men. Since the introduction of ICSI in the treatment of infertility, several controversies have arisen. Early reports illustrated that ICSI success is not influenced by sperm parameters such as concentration, morphology, or motility and only injection of immotile sperm influenced ICSI outcomes negatively.[1-5] With more research conducted in this field, it was shown that severe asthenozoospermia can adversely impact pregnancy rates when compared to cases with higher than 5% sperm motility.[6] Moreover, sperm morphology was similarly shown to negatively impact ICSI outcomes.[7,8]

There is mounting evidence to show that severe asthenozoospermia and cryptozoospermia can negatively affect fertilization and clinical pregnancy rates with ICSI.[9] These findings may be explained by the high probability of sperm DNA damage and chromosomal abnormalities in men with low sperm counts.[10] Furthermore, sperm DNA damage was found to be a predictor of negative ICSI outcomes independent of concentration, morphology or motility.[11-13] With these findings in mind, several researchers observed that ejaculated sperm is subject to more DNA damage than sperm acquired from the testis in both human and animal studies.[14,15] These findings prompted further research in the use of testicular sperm in ICSI and several early reports showed superior pregnancy and live births but lower miscarriage rates with the use of testicular rather than ejaculated sperm-ICSI in couples with severe oligozoospermia (spermatozoa count <5 million/ml) and cryptozoospermia (few sperm in the ejaculate that are identified only after centrifugation of semen sample).[9,16-18] These findings were challenged with a meta-analysis that failed to show a difference in ICSI pregnancy rates between testicular and ejaculated sperm-ICSI in cryptozoospermic couples.[19] Taking all of this into account, there is no robust evidence to show that severely oligozoospermic men have worse outcomes with ejaculate compared to testicular sperm-ICSI, even in absence of high DNA fragmentation. However, the evidence that severe oligozoospermia and sperm DNA damage impact the success rate of ICSI when ejaculated sperm are used remains.[9] In this study, we sought to evaluate TESA sperm retrieval rates and ICSI outcomes in men with severe oligozoospermia and cryptozoospermia.

Methods

Patients

Data were collected retrospectively from 88 consecutive, non-azoospermic, infertile men with idiopathic severe oligozoospermia (spermatozoa count <5 million/ml) or cryptozoospermia (few sperm in the ejaculate that are identified only after centrifugation of semen sample) who underwent TESA after one or more failed ICSI attempt(s) using ejaculated sperm between January 2011 and January 2018 at the OVO fertility clinic in Montreal, Canada. Patients were categorized into four groups according to sperm concentration: <5 and >1 million/ml (group 1), <1 and >0.1 million/ml (group 2), <0.1 million/ml (group 3), and cryptozoospermia (group 4). We excluded couples with advanced female age (>40 years). We also excluded couples with a correctable male factor (e.g., varicocele, semen infection).

Consent was not obtained from patients. The research and development scientific committee at OVO clinic reviewed our study and we acquired the approval as a quality control study. Also, we followed the Helsinki declaration principle.

Semen analysis was done using a microptic SCA (Sperm Class Analyzer, Microptic, Barcelona, Spain), with measurements sperm motility taken at 37 °C. All men were evaluated in our clinic with a thorough history, physical examination, and relevant laboratory testing. At our IVF center, TESA is done fresh the day before oocyte retrieval in keeping with our embryologist's preference. Before performing TESA-ICSI, every case was first reviewed by the clinical team (urologist, gynecologist, and embryologist). The nature of testicular sperm-ICSI was discussed with the patients. Moreover, we informed the couples about the potential benefits and risks of testicular sperm retrieval (bleeding, infection, pain, hypogonadism, unknown genetic and epigenetic risks). In regard to patients with cryptozoospermia included in our study, they were counselled for microdissection testicular sperm extraction (microTESE), but they elected to undergo TESA. Genetic testing (karyotype and Y-chromosome microdeletion) was done on all patients.

We collected the following variables in our study: patient and partner age, testicular volumes, serum follicle-stimulating hormone (FSH) level, total testosterone, total number of embryos transferred (ET), sperm retrieval rates, and clinical pregnancy rate (per embryo transfer). Live birth rate was not assessed because of loss of followup of most couples. We used the sperm chromatin structure assay (SCSA) to detect sperm DNA fragmentation (SDF) and treated men with moderately high SDF (>15.0% and <30%) or high SDF (>30.0%).

Testicular sperm retrieval

Testicular sperm retrieval was performed by TESA under local anesthesia and all procedures were performed by the same surgeon (AZ), as previously described.[20]

Statistical analysis

IBM Statistical Package for the Social Sciences (SPSS, version 20; SPSS Inc., IBM Corp., Armonk, NY, U.S.) was used to collect data and perform statistical analysis. Continuous variables were expressed as mean ± standard deviation (SD) and were assessed using one-way ANOVA. Fisher's exact test was used to compare dichotomous variables. A p-value <0.05 was considered statistically significant.

Results

We identified 88 patients that underwent testicular sperm ICSI. Although no early complications were reported, we could not adequately assess late TESA complications (e.g., chronic testicular pain, hypogonadism) because most of the patients did not return for followup after TESA. The baseline characteristics, sperm retrieval rates, and clinical pregnancy outcomes are shown in Table 1. The mean (± SD) male age

Table 1. Clinical characteristics, sperm retrieval outcomes and clinical pregnancy rates in couples with severe oligozoospermia and cryptozoospermia managed by TESA with ICSI

	Cryptozoospermia	<0.1 M	0.1–1 M	1–5 M	p
n	11	17	30	30	
Male age	34.6±1.5	37.9±1.5	35±1	39.1±1.4	0.052[a]
Female age	33.5±1.1	33±9.9	32±0.8	33.6±0.73	0.47[a]
Right testicular volume (mL)	16.1±0.9	15.1±0.9	14.7±0.8	16±0.7	0.55[a]
Left testicular volume (mL)	15.5±0.9	14.4±0.7	14.6±0.7	15.4±0.8	0.73[a]
FSH (IU/L)	12.4±3.3	10.3±1.7	10.2±1.9	11.1±1.7	0.91[a]
Total testosterone (nmol/L)	14.5±6.1	15.5±2.1	11.1±5.1	11.8±1.1	0.50[a]
Number of embryos transferred	2±0.32	1.7±0.24	1.95±0.3	1.7±0.16	0.73[a]
Successful sperm retrievals (%)	5/11 (45)	15/17 (88)	29/30 (97)	30/30 (100)	<0.001[b]
Pregnancy rate per ET (%)	0/5 (0)	4/11 (36)	13/20 (65)	9/21 (43)	0.05[b]

Values are means ± standard deviation. [a]Kruskal-Wallis one-way ANOVA on ranks. [b]Fisher's exact test. ET: embryo transfer; FSH: follicle-stimulating hormone; ICSI: intra-cytoplasmic sperm injection; TESA: testicular sperm aspiration.

was 37±7 years and the mean female age was 33±4 years. There was no significant difference in maternal or paternal age between the groups. The mean testicular volumes, serum FSH level, and number of ETs were comparable among the four groups, with no significant difference. We identified two men with genetic abnormalities: one with a 47 XYY- 45 X karyotype and another patient with a translocation (8:18). Bilateral TESA was done in two patients, with the remainder undergoing unilateral TESA. Sperm was recovered successfully in 90% (79/88) of the men overall and in 100% (30/30) of the men in group 1 (>1 to 5 million/ml), 97% (29/30) of the men in group 2 (0.1 to 1 million/ml), 88% (15/17) of the men in group 3 (<0.1 million/ml), and 45% (5/11) of the men in group 4 (cryptoozospermia). Sixty-five percent (57/88) of the couples had an ET. The overall clinical pregnancy rate per ET was 46% (26/57), with a mean of 1.8 ±0.3 embryos transferred per cycle. The clinical pregnancy rates (per ET) were 43% (9/21) in group 1, 65% (13/20) in group 2, 36% (4/11) in group 3, and 0% (0/5) in group 4.

There was a statistically significant difference in sperm retrieval rates between the groups ($p<0.05$), with less than 50% chance of finding sperm in men with cryptozoospermia. On the other hand, all men with a sperm concentration >1 million/ml had a successful sperm retrieval. Furthermore, we observed a lower pregnancy outcome in couples with cryptozoospermia ($p=0.05$).

Discussion

We performed a retrospective study on sperm retrieval outcomes and clinical pregnancy rates in couples with severe oligozoospermia and cryptozoospermia undergoing TESA with ICSI. We showed that, overall, TESA is associated with high sperm retrieval rates and acceptable ICSI pregnancy outcomes in men with severe oligozoospermia. However, in the subset of men with cryptozoosperma, TESA sperm retrieval rates and ICSI pregnancy rates were poor.

In our study, 90% of the men had successful sperm retrieval. Subgroup analysis showed that sperm were successfully recovered in 100% of the men in group 1 (>1 to 5 million/ml), 97% of the men in group 2 (0.1 to 1 million/ml), 88% of the men in group 3 (<0.1 million/ml), and 45% of the men in group 4 (cryptoozospermia). The poor sperm retrieval rate in the cryptozoospermic men in our study (45%) is comparable to that found by Alrabeeah et al (43%).[21] With the exception of sperm concentration, there was no significant difference among the subgroups in terms of baseline characteristics (age of couples, testicular volume, serum FSH level, number of ET), indicating a reasonable homogeneity in this cohort. Regarding the specific result of the two men with genetic abnormalities, both had successful sperm retrieval that resulted in pregnancy. The characteristics of the patient with a 47 XYY- 45 X karyotype were: <1 million/ml on semen analysis, 16 cc testicular volume, FSH 4 IU/L, total testosterone 10 nmol/L, one ET. The other patient with a translocation (8:18) had <5 million/ml on semen analysis, 20 cc testicular volume, and two ETs.

The reason for using testicular sperm varied according to the subgroup. The men in group 1 had prior ICSI failure with evidence of high sperm DNA fragmentation on DNA testing. The men in groups 2, 3, and 4 had failed one or more ICSI attempts using ejaculated sperm and were presumed to have sperm DNA fragmentation based on the association between sperm DNA fragmentation and standard sperm parameters (this could not be tested because the sperm DNA assay requires a minimum of 1 million cells/ml).[22]

The use of testicular sperm in the context of severe oligozoospermia is based on the rationale that: 1) sperm DNA fragmentation is common in men with severe oligozoospermia; 2) the level of sperm DNA fragmentation is significantly lower in testicular compared to ejaculated sperm; and 3) higher pregnancy rates are reported with the use of testicular compared to ejaculated sperm in men with high levels of sperm DNA fragmentation.[14,16,23,24] Similarly, experimental studies have shown that in animals with abnormal spermatogenesis, the passage of sperm with poor chromatin compaction through the epididymis results in sperm DNA fragmentation and impaired fertility potential.[15]

We have found a difference (albeit non-significant) in pregnancy rates per ET among the subgroups. The overall pregnancy rate in couples with severe oligozoospermia (groups 1, 2, and 3) was 46%. In contrast, no pregnancies were observed in the couples with cryptozoospermia. Although there is paucity of data in the literature on pregnancy outcomes with TESA-ICSI for men with severe oligozoospermia and cryptozoospermia, the available studies demonstrate favorable pregnancy rates in such patients using testicular sperm harvested by microTESE.[16,17,21] Mehta et al reported a pregnancy rate of 50% in men with severe oligozoospermia and high DNA fragmentation index (DFI) using testicular sperm retrieved by micro-TESE.[17] Moreover, Inal et al evaluated men with severe oligozoospermia who underwent micro-TESE and found a 36% clinical pregnancy rate.[25]

The men in our study were stratified based on their sperm concentration because it has been shown that the degree of severity of oligozoospermia may negatively impact ICSI outcomes.[9] The studies on cryptozoospermic men have shown conflicting results. Several studies have demonstrated that using testicular sperm is associated with higher pregnancy and live birth rates compared to ejaculated sperm in cryptozoospermic men.[26-29] These observations support the belief that testicular sperm has better quality than ejaculated sperm through two explanations: 1) the acquired sperm DNA damage during epididymal transit; and 2) semen samples in men with cryptozoospermia undergoes extensive processing to be used in ICSI, which can increase oxidative stress and results in

poor sperm quality, which in turn has negative impact on ICSI outcomes.[19] Conversely, other investigators did not show any differences in the ICSI outcomes using testicular vs. ejaculated sperm in men with cryptozoospermia.[19,30] Our data do not support the use of testicular sperm recovered by TESA in men with cryptoozospermia. We suspect that the poor outcomes in this study may be related to the retrieval method (TESA), as we have previously shown good sperm retrieval and pregnancy outcomes in these couples when microTESE-ICSI is performed.[21]

Conclusions

Our study indicates that TESA allows for high sperm retrieval rates and acceptable ICSI pregnancy rates in men with severe oligozoospermia. However, in our experience, TESA sperm retrieval rates were poor in cryptozoospermic men, suggesting that this sperm retrieval method is not optimal for this cohort. Moreover, ICSI outcomes were also poor in couples with cryptozoospermia. Certainly, there is a need for larger, well-designed, randomized, prospective studies to assess the value of sperm DFI and sperm retrieval techniques in men with severe oligozoospermia and cryptozoospermia. Moreover, such studies will determine which subgroups may benefit most from use of testicular sperm in ICSI.

Competing interests: Dr. Zini is a shareholder in Yad-Tech Nutraceutical. The remaining authors report no competing personal or financial interests related to this work.

This paper has been peer-reviewed.

References

1. Palermo G, Joris H, Devroey P, et al. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet* 1992;340:17-8. https://doi.org/10.1016/0140-6736(92)92425-F
2. Palermo G, Joris H, Derde MP, et al. Sperm characteristics and outcome of human-assisted fertilization by subzonal insemination and intracytoplasmic sperm injection. *Fertil Steril* 1993;59:826-35. https://doi.org/10.1016/S0015-0282(16)55867-1
3. Nagy ZP, Liu J, Joris H, et al. The result of intracytoplasmic sperm injection is not related to any of the three basic sperm parameters. *Hum Reprod* 1995;10:1123-9. https://doi.org/10.1093/oxfordjournals.humrep.a136104
4. Nijs M, Vanderzwalmen P, Vandamme B, et al. Fertilizing ability of immotile spermatozoa after intracytoplasmic sperm injection. *Hum Reprod* 1996;11:2180-5. https://doi.org/10.1093/oxfordjournals.humrep.a019073
5. Vandervorst M, Tournaye H, Camus M, et al. Patients with absolutely immotile spermatozoa and intracytoplasmic sperm injection. *Hum Reprod* 1997;12:2429-33. https://doi.org/10.1093/humrep/12.11.2429
6. Mitchell V, Rives N, Albert M, et al. Outcome of ICSI with ejaculated spermatozoa in a series of men with distinct ultrastructural flagellar abnormalities. *Hum Reprod* 2006;21:2065-74. https://doi.org/10.1093/humrep/del130
7. De Vos A, Van De Velde H, Joris H, et al. Influence of individual sperm morphology on fertilization, embryo morphology, and pregnancy outcome of intracytoplasmic sperm injection. *Fertil Steril* 2003;79:42-8. https://doi.org/10.1016/S0015-0282(02)04571-5
8. Van den Hoven L, Hendriks JC, Verbeet JG, et al. Status of sperm morphology assessment: An evaluation of methodology and clinical value. *Fertil Steril* 2015;103:53-8. https://doi.org/10.1016/j.fertnstert.2014.09.036
9. Strassburger D, Friedler S, Raziel A, et al. Very low sperm count affects the result of intracytoplasmic sperm injection. *J Assist Reprod Genet* 2000;17:431-6. https://doi.org/10.1023/A:1009413201849
10. De Braekeleer M, Dao TN. Cytogenetic studies in male infertility: A review. *Hum Reprod* 1991;6:245-50. https://doi.org/10.1093/oxfordjournals.humrep.a137315
11. Zini A, Boman JM, Belzile E, et al. Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: Systematic review and meta-analysis. *Hum Reprod* 2008;23:2663-8. https://doi.org/10.1093/humrep/den321
12. Zhao J, Zhang Q, Wang Y, et al. Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization/intracytoplasmic sperm injection: A systematic review and meta-analysis. *Fertil Steril* 2014;102:998-1005; discussion e1008. https://doi.org/10.1016/j.fertnstert.2014.06.033
13. Oleszczuk K, Giwercman A, Bungum M. Sperm chromatin structure assay in prediction of in vitro fertilization outcome. *Andrology* 2016;4:290-6. https://doi.org/10.1111/andr.12153
14. Greco E, Scarselli F, Iacobelli M, et al. Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa. *Hum Reprod* 2005;20:226-30. https://doi.org/10.1093/humrep/deh590
15. Suganuma R, Yanagimachi R, Meistrich ML. Decline in fertility of mouse sperm with abnormal chromatin during epididymal passage as revealed by ICSI. *Hum Reprod* 2005;20:3101-8. https://doi.org/10.1093/humrep/dei169
16. Esteves SC, Sanchez-Martin F, Sanchez-Martin P, et al. Comparison of reproductive outcome in oligozoospermic men with high sperm DNA fragmentation undergoing intracytoplasmic sperm injection with ejaculated and testicular sperm. *Fertil Steril* 2015;104:1398-1405. https://doi.org/10.1016/j.fertnstert.2015.08.028
17. Mehta A, Bolyakov A, Schlegel PN, et al. Higher pregnancy rates using testicular sperm in men with severe oligospermia. *Fertil Steril* 2015;104:1382-7. https://doi.org/10.1016/j.fertnstert.2015.08.008
18. Negri L, Patrizio P, Albani E, et al. ICSI outcome is significantly better with testicular spermatozoa in patients with necrozoospermia: A retrospective study. *Gynecol Endocrinol* 2014;30:48-52. https://doi.org/10.3109/09513590.2013.848427
19. Abhyankar N, Kathrins M, Niederberger C. Use of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with cryptozoospermia: A meta-analysis. *Fertil Steril* 2016;105:1469-75. https://doi.org/10.1016/j.fertnstert.2016.02.013
20. Al-Malki AH, Alrabeeah K, Mondou E, et al. Testicular sperm aspiration (TESA) for infertile couples with severe or complete asthenozoospermia. *Andrology* 2017;5:226-31. https://doi.org/10.1111/andr.12317
21. Alrabeeah K, Wachter A, Phillips S, et al. Sperm retrieval outcomes with microdissection testicular sperm extraction (micro-TESE) in men with cryptozoospermia. *Andrology* 2015;3:462-6. https://doi.org/10.1111/andr.12000
22. Moskovtsev S, Willis J, White J, et al. Sperm DNA damage: Correlation to severity of semen abnormalities. *Urology* 2009;74:789-93. https://doi.org/10.1016/j.urology.2009.05.043
23. Esteves SC, Roque M, Bradley CK, et al. Reproductive outcomes of testicular vs. ejaculated sperm for intracytoplasmic sperm injection among men with high levels of DNA fragmentation in semen: Systematic review and meta-analysis. *Fertil Steril* 2017;108:456-67.e1. https://doi.org/10.1016/j.fertnstert.2017.06.018
24. Herrero MB, Lusignan MF, Son WY, et al. ICSI outcomes using testicular spermatozoa in non-azoospermic couples with recurrent ICSI failure and no previous live births. *Andrology* 2019;7:281-7. https://doi.org/10.1111/andr.12591
25. Inal HA, Kahyaoglu I, Turkkani A, et al. Retrospective comparison of intracytoplasmic sperm injection outcomes of sperm retrieved from a testicular biopsy and freshly ejaculated semen in oligozoospermia. *Rev Int Androl* 2018;16:131-6. https://doi.org/10.1016/j.androl.2017.06.006
26. Ben-Ami I, Raziel A, Strassburger D, et al. Intracytoplasmic sperm injection outcome of ejaculated vs. extracted testicular spermatozoa in cryptozoospermic men. *Fertil Steril* 2013;99:1867-71. https://doi.org/10.1016/j.fertnstert.2013.02.025
27. Bendikson KA, Neri QV, Takeuchi T, et al. The outcome of intracytoplasmic sperm injection using occasional spermatozoa in the ejaculate of men with spermatogenic failure. *J Urol* 2008;180:1060-4. https://doi.org/10.1016/j.juro.2008.05.025
28. Hauser R, Bibi G, Yogev L, et al. Virtual azoospermia and cryptozoospermia — fresh/frozen testicular or ejaculate sperm for better IVF outcome? *J Androl* 2011;32:484-90. https://doi.org/10.2164/jandrol.110.011353
29. Cui X, Ding P, Gao G, et al. Comparison of the clinical outcomes of intracytoplasmic sperm injection between spermatozoa retrieved from testicular biopsy and from ejaculate in cryptozoospermia patients. *Urology* 2016;102:106-10. https://doi.org/10.1016/j.urology.2016.08.071
30. Amirjannati N, Heidari-Vala H, Akhondi MA, et al. Comparison of intracytoplasmic sperm injection outcomes between spermatozoa retrieved from testicular biopsy and from ejaculation in cryptozoospermic men. *Andrologia* 2012;44Suppl. 1:704-9. https://doi.org/10.1111/j.1439-0272.2011.01253.x

Correspondence: Dr. Armand Zini, St. Mary's Hospital, Montreal, QC, Canada; ziniarmand@yahoo.com