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Original Research Article

Fertility outcomes in microdissection testicular sperm extraction for non-obstructive azoospermia

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ABSTRACT

Background: The objective of this study was to evaluate outcome of micro dissection testicular sperm extraction (micro TESE) and intracytoplasmic sperm injection (ICSI) for treatment of non-obstructive azoospermia (NOA).
Methods: We retrospectively analysed data of 96 consecutive patients with clinical NOA who were treated with micro TESE by single surgeon, between January 2022 and December 2022, in Lifeline superspeciality hospital Adoor, Kerela. Embryological and clinical outcomes were demonstrated based on ICSI-IVF cycles using fresh or frozen sperms, different etiologies of NOA and various counts of sperms retrieved.
Results: 96 men underwent micro TESE and 72.9% (70/96) of them had sperms retrieved. ICSI performed in 64 couples. Of those, 41 reached the stage of embryo transfer (ET). Of the couples who underwent embryo transferred, 18 (43%) resulted in biochemical pregnancies and 7 (17%) clinical pregnancies. There was a significant difference in the testicular volume and serum FSH levels between micro-TESE positive and negative groups (p=0.000). Retrieval rates were higher in group of men with normal testicular volume and FSH<12. Clinical pregnancy rate was around 11% in couples who had sperms retrieved by micro TESE. The sperm retrieval rates were higher in men with age <40 years. Similarly younger the female age more was the pregnancy rate. Out of various etiologies idiopathic NOA and Klinefelter syndrome had better sperm retrieval rate in our study which was statistically significant. Total fertilization rate and blasts rates were 79.7% and 51.6% out of ICSI.
Conclusions: Microdissection testicular sperm extraction is an effective treatment for NOA with higher rate of sperm retrieval and pregnancy rate. The increasing success rates over several years indicate the importance of surgical skill and laboratory staff experience.

Keywords: FSH levels, Microdissection testicular sperm extraction, Non-obstructive azoospermia

INTRODUCTION

Non-obstructive azoospermia (NOA) is caused by testicular failure, which may be primary (testicular) or secondary to hypothalamic-pituitary dysfunction (pre-testicular). It constitutes 60-70% of all cases of azoospermia.[1]

NOA is heterogenous condition, with impaired spermatogenesis resulting from hypospermatogenesis, maturation cell arrest or sertoli cell-only syndrome (SCOS).[2,3] Klinefelter's syndrome (KS) and Y-chromosome microdeletions represents the most common congenital causes of NOA.[4] However, most NOA patients have the unknown cause of their azoospermia.[5] Acquired causes of NOA include testicular torsion, mumps orchitis, cryptorchidism and iatrogenic causes such as medications, chemotherapy and radiotherapy.[6]

Fertility management of patients with NOA relies on surgical sperm retrieval techniques. Devroey et al were the first to performed conventional testicular sperm extraction (TESE) for NOA patients in 1995.[7] In the past 20 years, microdissection testicular sperm extraction (micro-TESE)

has gradually become a popular surgical technique with a high SRR and low tissue loss.[8,] Microdissection TESE (micro-TESE) followed by intracytoplasmic sperm injection (ICSI) was first described in 1999 by Schlegel et al.[9] Recent data suggest that testicular volume >12.5 ml is associated with a higher sperm retrieval rate SRR and smaller testicular volume is associated with worse prognosis.[10,11]

Surgeon skill and experience (particularly when micro-TESE is used) has shown improved outcomes.[12] Furthermore, different tissue processing methods, the time, skills and effort dedicated to the identification of spermatozoa in the testicular specimen may greatly affect the sperm retrieval rates.[13]

METHODS

We conducted a retrospective study analysis of 96 men diagnosed with NOA at lifeline superspeciality center, between January 2022 to December 2022, who underwent micro TESE by single surgeon to be used for ICSI. Azoospermia was confirmed for all the patients on at least two different occasions by testing the centrifuged ejaculates according to World Health Organisation guidelines.[14] Out of 96 men, 43 had testosterone level <300 ng/dl and were treated with medication to optimize endogenous testosterone levels prior to micro TESE. Aromatase inhibitors (1 mg anastrozole daily), urinary human chorionic gonadotrophin (urinary derived hCG, 10000 IU once weekly intramuscularly) were used for at least 2 months before micro-TESE. Patients underwent a complete clinical evaluation to determine the etiology of azoospermia including age, detailed history and clinical examination. Testicular ultrasound, hormonal evaluation (FSH and testosterone), karyotyping and Y chromosome microdeletion analysis were done. Idiopathic NOA was diagnosed by excluding known causes of NOA. Patients with history of micro TESE, history of radiation and chemotherapy were excluded from the study. All patients underwent micro TESE. The same surgeon performed all micro TESE procedures.

In addition, female's ovarian reserve would be assessed in detail before the eventual decision on conduction of micro-TESE surgery to reduce the risk of cycle cancellation due to female factors.

Micro-TESE

Briefly, procedures were performed under spinal anesthesia. A floor-standing operating microscope (OPMI Vario/S88 System, Karl Zeiss, India) was used throughout the operations. After adequate skin disinfecting and draping, the scrotal skin was stretched over the anterior surface of the testis, and 2.5 cm transverse incision was placed. This incision was carried out through the dartos muscle and tunica vaginalis. The tunica was opened and its bleeders were cauterized. The testis was delivered extravaginally and the tunica albuginea was examined to select the site of injection. A single large equatorial incision covering approximately 270° of the circumferences of the testis was made on an avascular area in tunica albuginea under ×6-8 magnification, and the testicular parenchyma was widely exposed. A tiny testicular fragment of approximately 5×5 mm was excised from the medium testicular pole and placed in Bouin's fixative for histopathology examination. This amount of excised tissue yields a sufficient number of seminiferous tubules (>20 cross-sections) to perform an adequate quantitative analysis of the specimen.[15] Dissection of the testicular parenchyma was undertaken at ×16-25 magnification in search for enlarged seminiferous tubules which are more likely to contain germ cells as originally described by Schlegel.[9] The superficial and deep testicular regions were examined, as needed and operating microscope-guided testicular regions were examined, as needed, and operating microscope-guided testicular biopsies were performed at the upper, medium, and lower testicular poles. The excised specimens were placed into the center well of Petri dishes containing buffered sperm medium in room temperature. Specimens were washed grossly to remove blood clots and sent to the IVF laboratory immersed in HEPES-buffered sperm medium kept at room temperature. The surgeon was promptly informed about the results of initial microscopic examination of each extracted specimen, which were delivered by the embryologist in approximately 3-5 minutes. This was possible since the operating room and the IVF lab were located side-by-side with a communicating pass-through window. The albuginea was closed using continuous non-absorbable 6-0 nylon suture. Following hemostasis, the tunica vaginalis was closed in a running fashion using 5-0 absorbable suture. Then, dartos muscle was closed with interrupted absorbable sutures. Last, the skin was closed with continuous subcuticular 5-0 Vycril suture, and a fluffy-type dressing and scrotal supporter were placed. The procedures were carried out at the contralateral testicle, as described earlier, when an insufficient number or no sperm have been found at initial laboratory examination. Patients were discharged 1 day after surgery. Prior to discharge patients were examined to rule out scrotal hematoma.

Ovarian stimulation and oocyte retrieval

In couple testicular sperm was retrieved undergoing a synchronous micro-TESE-ICSI procedure, female partners underwent ovarian stimulation using recombinant FSH or hMG combined GnRH antagonists.[16] Oocyte-cumulus complexes were recovered 36 hours after administering 500 µg of recombinant hCG.

ICSI procedure, embryo culture, and transfer

Next ICSI and fertilization assessment were performed as previously described by Liu.[16] Fertilization rates were expressed as the percentage of oocytes with two distinct pronuclei per injected metaphase II oocytes. Embryos were scored by their morphological appearance according

to the society for assisted reproductive technology scoring system.[17] Normally cleaving embryos with ≥5 cells and ≤20% fragmentation were considered eligible for transfer. Up to two embryos were transferred into the uterine cavity on day 5 in next cycle.

Pregnancy follow-up

Pregnancy was diagnosed by elevated serum hCG levels (≥25 IU/l) 14 days after embryo transfer. Clinical pregnancy was defined as a visible gestational sac at transvaginal ultrasound 4-5 weeks after embryo transfer. Pregnancy loss was defined as the loss of a clinical pregnancy before 28 weeks of gestation.

RESULTS

96 men underwent micro TESE and 72.9% (70/96) of them had sperm retrieved. ICSI performed in 64 couples. Of those, 41 reached the stage of embryo transfer (ET). Of the couples who underwent embryo transferred, 18 (43%) resulted in biochemical pregnancies and 7 (17%) clinical pregnancies (Table 1).

Table 1: Pregnancy outcome of micro TESE and ICSI.

Outcomes		Frequency	Percentage
Biochemical pregnancy	Positive	18	43.9
	Negative	23	56.1
Clinical pregnancy	Positive	7	17.1
	Negative	34	82.9
Total embryo transferred		41	100

Table 2: Total sperm retrieval.

Outcomes		Frequency	Percentage
Total sperm retrieval	Positive	70	72.9
	Negative	26	27.1
Total		96	100

Table 3: Association between volume of testis and sperm retrieval rate.

Volume of testis	Micro-TESE findings		Total	P value
	Positive	**Negative**		
Normal volume	54 (77.1%)	7 (26.9%)	61 (63.5%)	0.000
Low volume	16 (22.9%)	19 (73.1%)	35 (36.5%)	
Total	70 (100%)	26 (100%)	96 (100%)	

There was a significant difference in the testicular volume and serum FSH levels between micro-TESE positive and negative groups (p=0.000). Retrieval rates were higher in group of men with normal testicular volume (Table 3) and FSH<12 (Table 4).

Table 4: Association between sperm retrieval and FSH values.

FSH	Micro-TESE findings		P value
	Positive	**Negative**	
Mean	7.84±5.83	16.41±12.46	0.000

Table 5: Pregnancy rate in micro-TESE with respect to age of husband.

Age of husband	Micro-TESE findings	Clinical Pregnancy		Total	P value
		Positive	**Negative**		
21 to 30 years	Positive	0	17	17	NA
	Negative	0	5	5	
31 to 40 years	Positive	6	36	42	0.440
	Negative	1	14	15	
>40 years	Positive	0	11	11	NA
	Negative	0	6	6	
Total		7	89	96	

Table 6: Association between sperm retrieval related to age.

Age group (husband)	Micro-TESE findings		Total	P value
	Positive	**Negative**		
21 to 30 years	17 (24.3%)	5 (19.2%)	22 (22.9%)	0.667
31 to 40 years	42 (60.0%)	15 (57.7%)	57 (59.4%)	
>40 years	11 (15.7%)	6 (23.1%)	17 (17.7%)	
Total	70 (100%)	26 (100%)	96 (100%)	
Mean age	34.94±5.45	35.96±6.86	P value 0.451	

Table 7: Association between Causes of NOA (Klinefelter syndrome, AZFc microdeletion and idiopathic and sperm retrieval.

Variables		Sperm retrieval		Total	P value
		Positive	Negative		
Klinefelter	Yes	3	8	11	0.001
	No	67	18	85	
Y-Chromosome	AZFc deletion	15	8	23	0.244
	No deletion	55	18	73	
Idiopathic	Yes	52	10	62	0.002
	No	18	16	34	
Total		70 (100%)	26 (100%)	96 (100%)	

Clinical pregnancy rate was around 11% in couples who had sperms retrieved by micro TESE. The sperm retrieval rates were higher in men with age <40 years. Similarly younger the female age more was the pregnancy rate (Table 5 and 6).

In the present study, the overall SSR rate of all NOA patients was 72.9%. Out of various etiologies sperm retrieval were higher among idiopathic NOA (83.87%) and Klinefelter syndrome (27.2%) in our study which was statistically significant (Table 7).

Table 8: Fertilization rate and blasts rate out of total ICSI done.

Fertilization, blast formation		Frequency	Percentage
Fertilization	Yes	51	79.7
	No	13	20.3
Blasts formation	Yes	33	51.6
	No	31	48.4
Total		64	100

Total fertilization rate and blasts rates were 79.7% and 51.6% out of ICSI (Table 8).

DISCUSSION

Non-obstructive azoospermia (NOA) is one of the most difficult conditions to deal with pertaining to male infertility. It leads to disruption of spermatogenesis. Sperm retrieval (SR) coupled with intracytoplasmic sperm injection (ICSI) is the only valid option for such patients seeking self-parentage. Literature quotes retrieval rates ranging from 35% to 77% for micro-TESE.[18,19] In our 72.9% (70/96) of them had sperm retrieval. Therefore, micro-TESE could be a viable option as first line treatment in patients with non-obstructive azoospermia.

The current study also showed that male partner age dint have significant effect on micro-TESE sperm retrieval success rate. Previous studies have been inconsistent in their findings regarding the effect of age on micro-TESE success whereas, a study from Iran stated that SRR may significantly decrease in older men in comparison with their younger counterpart.[20-22]

The current study showed probable inverse relation between serum FSH and micro-TESE SRR.SSR were higher in mean with mean FSH<12. This is in line with study by Saheli et al.[23] However study by Chen, showed serum FSH level should not preclude patients from micro-TESE procedure.[24]

In addition, a previous study showed that the strongest predictor of the success rate of sperm retrieval was testicular histopathology which we did not examine in the present study.[25] However, performing a biopsy before surgery is not recommended. One reason is that spermatogenesis may be active even in the unsuccessful pathologies.

The reported SRR of micro-TESE in KS syndrome was between 21-72%.[20] The prevalence of KS and SRR rate, in the current study were 11.45% and 27% respectively. Therefore, it is reasonable to offer micro-TESE to all male partners with KS syndrome, despite its low success rate.

Fertilization rate and clinical pregnancy rates were 79.7%, 17.1% respectively and are in patients with the study by Ghanem et al found to be 54.2%, 23.1% respectively among NOA patients.[26]

In the present study, the overall SRR rate of all NOA patients were 72.9%. in addition, the sperm retrieval was most successful in the idiopathic group (83.87%), followed by YCMD (65.2%) and KS (27.2%) is contradictory to the study by Gao et al it was least successful in idiopathic group (31.22%), followed by KS (48.65%), YCMD (60.87%).[27]

The main limitations of the study were the small sample size; incomplete participant's information; lack of testicular biopsy for confirmed pathology.

CONCLUSION

Microdissection testicular sperm extraction is an effective treatment for NOA with higher rate of sperm retrieval and

pregnancy rate. The increasing success rates over several years indicate the importance of surgical skill and laboratory staff experience.

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REFERENCES

1. Sharlip ID, Jarow JP, Belker AM, Lipshultz LI, Sigman M, Thomas AJ, et al. Best practice policies for male infertility. Fertil Steril. 2002;77(5):873-82.
2. Tournaye H, Krausz C, Oates RD. Concepts in diagnosis and therapy for male reproductive impairment. Lancet Diabetes Endocrinol. 2017;5(7):554-64.
3. Pan MM, Hockenberry MS, Kirby EW, Lipshultz LI. Male infertility diagnosis and treatment in the era of in vitro fertilization and intracytoplasmic sperm injection. Med Clin North Am. 2018;102(2):337-47.
4. Corona G, Pizzocaro A, Lanfranco F, Garolla A, Pelliccione F, Vignozzi L, et al. Sperm recovery and ICSI outcomes in Klinefelter syndrome: a systematic review and meta-analysis. Hum Reprod Update. 2017;23(3):265-75.
5. Dohle GR, Halley DJ, Van Hemel JO, van den Ouwel AM, Pieters MH, Weber RF, et al. Genetic risk factors in infertile men with severe oligozoospermia and azoospermia. Hum Reorod. 2002;17(1):13-6.
6. Krausz C. Male infertility: pathogenesis and clinical diagnosis. Best Pract Res Clin Endocrinol Metab. 2011;25(2):271-85.
7. Devroey P, Liu J, Nagy Z, Goossens A, Tournaye H, Camus M, et al. Pregnancies after testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia. Hum Reprod. 1995;10(6):1457-60.
8. Bernie AM, Mata DA, Ramasamy R, Schlegel PN. Comparison of microdissection testicular sperm extraction, conventional testicular sperm extraction, and testicular sperm aspiration for non- obstructive azoospermia: a systematic review and meta-analysis. Fertil Steril. 2015;104:1099-103.
9. Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. Hum Reprod. 1999;14(1):131-5.
10. Arshad MA, Majzoub A, Esteves SC. Predictors of surgical sperm retrieval in non-obstructive azoospermia: summary of current literature. Int Urol Nephrol. 2020;52(11):2015-38.
11. Tsujimura A, Matsumiya K, Miyagawa Y, Takao T, Fujita K, Koga M, et al. Prediction of successful outcome of microdissection testicular sperm extraction in men with idiopathic nonobstructive azoospermia. J Urol. 2004;172(5):1944-7.
12. Ishikawa T, Nose R, Yamaguchi K, Chiba K, Fujisawa M. Learning curves of microdissection testicular sperm extraction for nonobstructive azoospermia. Fertil Steril. 2010;94(3):1008-11.
13. Ramasamy R, Reifsnyder JE, Bryson C, Zaninovic N, Liotta D, Cook CA, et al. Role of tissue digestion and extensive sperm search after microdissection testicular sperm extraction. Fertil Steril. 2011;96(2):299-302.
14. WHO. WHO laboratory manual for the examination and processing of human semen. 6th ed. WHO: Geneva; 2023.
15. Esteves SC, Glina S. Recovery of spermatogenesis after microsurgical subinguinal varicocele repair in azoospermia men based on testicular histology. Int Braz J Hum Reprod Sci. 2005;5:233-43.
16. Liu H, Liu J Chen S, Kang X, Du H, Li L. Elevated incidence of monozygotic twinning is associated with extended embryo culture, but not with zona pellucida manipulation or freeze- thaw procedure. Fertil Steril. 2018;109:1044-50.
17. Racowsky C, Stern JE, Gibbons WE, Behr B, Pomeroy KO, Biggers JD. National collection of embryo morphology data into society for assisted reproductive technology clinic outcomes reporting system: associations among day 3 cell number, fragmentation and blastomere asymmetry, and live birth rate. Fertil Steril. 2011;95:1985-9.
18. Silber SJ. Microsurgical TESE and the distribution of spermatogenesis in non-obstructive azoospermia. Hum Reprod. 2000;15:2278-84.
19. Esteves SC, Miyaoka R, Agarwal A. Sperm retrieval techniques for assisted reproduction. Int Braz J Urol. 2011;37:570-83.
20. Sabbaghian M, Modarresi T, Hosseinifar H, Hosseini J, Farrahi F, Dadkhah F, et al. Comparison of sperm retrieval and intracytoplasmic sperm injection outcome in patients with and without Klinefelter syndrome. Urology. 2014;83:107-10.
21. Ramasamy R, Trivedi NN, Reifsnyder JE, Palermo GD, Rosenwaks Z, Schlegel PN. Age does not adversely affect sperm retrieval in men undergoing microdissection testicular sperm extraction. Fertil Steril. 2014;101:653-5.
22. Karamazak S, Kizilay F, Bahceci T, Semerci B. Do body mass index, hormone profile and testicular volume effect sperm retrieval rates of microsurgical sperm extraction in the patients with nonobstructive azoospermia? Turk J Urol. 2018;44:202-7.
23. Salehi P, Derakhshan-Horeh M, Nadeali Z, Hosseinzadeh M, Sadeghi E, Izadpanahi MH, et al. Factors influencing sperm retrieval following testicular sperm extraction in nonobstructive azoospermia patients. Clin Exp Reprod Med. 2017;44:22-7.
24. Chen SC, Hsieh JT, Yu HJ, Chang HC. Appropriate cut-off value for follicle-stimulating hormone in azoospermia to predict spermatogenesis. Reprod Biol Endocrinol. 2010;8:108-11.
25. Abdel Raheem A, Garaffa G, Rushwan N, De Luca F, Zacharakis E, Abdel Raheem T, et al. Testicular histopathology as a predictor of a positive sperm

retrieval in men with non-obstructive azoospermia. BJU Int. 2013;111:492-9.
26. Ghanem M, Bakr NI, Elgayaar MA, EI Mongy S, Fathy H, Ibrahim AH. Comparison of the outcome of intracytoplasmic sperm injection in obstructive and non-obstructive azoospermia in the first cycle: a report of case series and meta-analysis. Int J Androl. 2005;28(1):16-21.
27. Gao S, Yang X, Xiao X, Yin S, Guan Y, Chen Y. Outcomes and affecting factors for ICSI and micro TESE treatments in nonobstructive azoospermia patients with different etiologies: a retrospective analysis-Front. Endocrinol. 2022;13:1006208.

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