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Factors determining the sperm retrieval rate in fresh versus salvage micro-TESE: a comparative cohort study

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Abstract

Purpose We studied prospectively the role of FSH, testis volume, age, duration of infertility, hormonal therapy, varicocelectomy and testicular histopathology in determining the sperm retrieval rate (SRR) in fresh versus salvage micro-TESE.

Methods Our study analysed retrospectively the prospectively maintained database of 330 NOA patients who underwent micro-TESE either 1st or 2nd or 3rd timers from January 2017 to August 2018 from the Andrology Clinic of a specialized IVF centre. They were divided equally into 3 groups, group (1) were 1st timers, group (2) were 2nd timers and group (3) were 3rd timers, respectively.

Results Interestingly, our study demonstrated a positive correlation between high FSH level and favourable micro-TESE outcome in 1st timers who underwent micro-TESE (p 0.015). Additionally, our study revealed a positive correlation between age and favourable micro-TESE outcome in 2nd timers who underwent micro-TESE (p 0.031). Further, our study revealed a positive correlation between right testicular volume and favourable micro-TESE outcome in 1st timers who underwent micro-TESE (p 0.023). Eventually, there was a significant positive correlation between histopathology which was mainly sertoli cell only syndrome and favourable micro-TESE outcome in 1st timers micro-TESE (p 0.001).

Conclusion Our study demonstrates that sperms can be retrieved despite high FSH level, and preoperative histopathology is not essential in predicting SRR in NOA patients. Further, age can be considered a good prognostic factor in patients who undergo salvage micro-TESE for the 2nd time.

Keywords Non-obstructive azoospermia · Fresh micro-TESE · Salvage micro-TESE · Non-surgical parameters (FSH, testis volume, age, duration of infertility, hormonal therapy) · Surgical parameters (varicocelectomy, testicular histopathology) · Sperm retrieval hope

Introduction

Testicular sperms can be retrieved in non-obstructive azoospermia (NOA) using a conventional open testicular sperm extraction (TESE) technique in 10–50% or testicular sperm aspiration (TESA) in 10–20% [1]. A number of historical studies suggest that there may be a correlation between testicular histology and the chances of finding sperms, although using the micro-dissection TESE (micro-TESE) technique, testicular histology does not appear to be a significant factor in predicting the outcome [2]. Micro-TESE was first introduced by Schlegel and Li and appears to be safe, resulting in minimal complications, together with a higher yield of sperms than conventional TESE and TESA [3–5]. A management dilemma occurs when patients with NOA have undergone negative sperm retrieval with less exhaustive sperms retrieval techniques, such as TESA and

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multiple biopsy TESE or single biopsy TESE in an attempt to retrieve sperms for intracytoplasmic sperm injection (ICSI). Sperms may not be found in these cases and they remain in a dilemma as whether to proceed to a micro-TESE to maximize the chances of finding sperms or potentially go down the route of adoption or use donor sperms [2]. Currently, there exists very little published data on the outcome of patients in this difficult group [2, 4, 6–8]. We aimed in this study to investigate the likelihood of sperms retrieval in repeated micro-TESE in NOA patients with a former unsuccessful sperm recovery attempt, together with evaluation of preoperative non-surgical (testicular volume, FSH level, age of the patients, duration of marriage, hormonal therapy) and surgical (varicocelectomy and previous histopathology) parameters in determining the sperm retrieval rates in these patients.

Methods

Study design and participants

Three hundreds and thirty NOA patients who underwent micro-TESE either 1st or 2nd or 3rd timers from January 2017 to August 2018 from the Andrology Clinic of a specialized IVF centre were recruited for this study whose databases were maintained prospectively at this centre. Their databases were divided equally into 3 groups, group (1) were 1st timers, group (2) were 2nd timers and group (3) were 3rd timers, respectively, and were analysed retrospectively. The Research Ethical Committee of Beni-Suef Faculty of medicine approved the study design which conforms to Helsinki declaration 1964. Before enrollment into the micro-TESE procedure, all the participants agreed to the study design and signed a written consent to allow us to use their data for analysis with their confidentiality guaranteed.

Evaluation of the study participants

Prospectively maintained databases of men with diagnosis of NOA were retrieved in this study. These men underwent micro-TESE and their prospectively maintained database were reviewed retrospectively for the sperm retrieval rates in relation to preoperative non-surgical and surgical parameters.

All the patients were subjected to the following

All participants were evaluated for full fertility history and genital examination that was done to detect varicocele and was confirmed by scrotal duplex (SONOLINE G40, Diagnostic Ultrasound Systems, Manufactured by Siemens AG, Eriangen, Germany). The testis was measured by scrotal ultrasound using the same sonar machine (1–2 ml atrophied, 2–8 ml small volume, 8–12 ml moderate, > 12 ml normal), in addition to Prader's orchidometer.

All the patients had their serum hormone levels measured using chemiluminescence immunoassay (CLIA) technique, with values in the range 1.5–14 mIU/ml for FSH, 1.5–8 mIU/ml for LH, 2.5–17 ng/ml for prolactin, 2.4–8.3 ng/ml for total testosterone and 20–47 pg/ml for estradiol were taken as normal. A fasting morning serum sample for basal hormones determination was obtained prior to micro-TESE. All assays were performed using Cobas E411 immunoassay analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Besides, Giemsa Karyotype was used for standard cytogenetic procedure in all patients by analysis of at least 20 G-banded metaphases from a peripheral blood lymphocyte culture, and in all cytogenetically normal cases, molecular screening for Yq microdeletions was carried out on DNA extracted from peripheral blood using PCR [9]. Micro-TESE was performed under general anaesthesia with the patient in a supine position. A floor-standing operating microscope (Leica M500; Leica microsystems Pty Ltd, Gladesville, NSW, Australia) was used throughout the procedures. Different senior Andrologists with expertise in microsurgery were responsible to setup and perform the technique of micro-TESE [10, 11]. Testicular tissues were observed under optical magnification X24 (dual-headed binocular tube and eyepieces 200, 300, 350-mm objective lens, motorized foot-operated zoom system). If no morphologically dilated tubules were observed, the incision was extended and blunt dissection performed between the septa of the testicular parenchyma to expose multiple areas. Copious irrigation of the field with ringer's lactate solution was carried out to prevent blood from obscuring the field, and multiple samples were taken from the most dilated tubules.

If there was no morphological difference in the appearance of testicular tissue, the samples were taken randomly, and a testicular fragment that is representative of the predominant tissue at microscopic examination during surgery was used for histopathological evaluation which was done for the 1st timers who underwent micro-TESE only, as the 2nd and 3rd timers had their histopathological patterns. If spermatozoa were not found, the contralateral testis was exposed via the same technique. Testicular tissues were taken in a petri dish 1 ml HEPES-buffered sperm medium (Ham's F10 medium, Gibco BRL, Grand Island, NY, USA) and testicular biopsies were minced using sterile glass slides and shredded with 2 jeweler forceps's under an Olympus stereo microscope (SZ-PT, Tokyo, Japan) to separate individual tubules and then examined immediately under an inverted microscope (Olympus IMT2) with Hoffman optics modulation (×400) for the presence of testicular spermatozoa in the entire petri dish.

Inclusion criteria of the patients

Patients with diagnosis of NOA and testicular volume < 15 ml, together with a normal ejaculate volume and alkaline pH were included in this study. In addition, cases with high FSH level (> 8 mIU/ml) who usually represent the majority of NOA patients together with patients with Klinefelter syndrome (KF) were also included in this study.

Exclusion criteria of the patients

Patients who suffer from severe systemic illness (end-stage renal disease; liver cell failure; congestive heart failure), cryptozoospermia, complete retrograde ejaculation, obstructive azoospermia (e.g. distended epididymides; impalpable vasa deferentia; vasectomy; findings suggestive of ejaculatory duct obstruction such as palpable seminal vesicles on digital rectal exam, hypospermia < 1.5 ml ejaculate or acidic semen pH; and dilated ejaculatory ducts or absent seminal vesicles on TRUS), NOA due to hypogonadotropic hypogonadism, bilateral intra-abdominal cryptorchidism, AZF Yq micro-deletion involving the sub-regions *a* or *b* and patients with abnormal karyotypes other than KF were excluded from this study. Patients receiving anabolic steroids and/or exogenous testosterone therapy within at least 6 months prior to the time of surgery or those who received gonadotoxic medications or NOA patients with testis volume > 15 ml were also excluded. Finally, we excluded all patients with previous history of favourable micro-TESE outcomes in the 2nd and 3rd timer patients either done at our centre or any other one.

Statistical methods

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 25. Data were summarized using median and interquartile range in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Mann–Whitney tests [12]. For comparing categorical data, chi-square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5 [13]. *P*-values < 0.05 were considered as statistically significant.

Result

Our study demonstrated that the mean ages of the patients in the 3 groups were 38.95 ± 7.77, 39.24 ± 7.69, 43.22 ± 7.61 years old, respectively (Table 1). This difference was of high statistical significance. The mean durations of infertility of the patients in the 3 groups were 8.14 ± 5.5, 8.77 ± 6.31, 12.33 ± 7.33 years, respectively (Table 1). This difference was of high statistical significance. Further, the mean FSH levels of the patients in the 3 groups were 17.86 ± 13.72, 17.77 ± 13.44, 25.11 ± 16.22 mIU/ml, respectively (Table 1). This difference was also of high statistical significance. Interestingly, our study demonstrated a positive correlation between high FSH level and favourable micro-TESE outcome in 1st timers who underwent micro-TESE (Table 1). Additionally, our study revealed a positive correlation between age and favourable micro-TESE outcome in 2nd timers who underwent micro-TESE (Table 1). Moreover, our study revealed that the majority of the patients in the 3 groups were occupying non-risky jobs [90 (82%), 72 (65.5%), 65 (61.3%), respectively], while the remainders were occupying risky jobs (e.g. prolonged exposure to heat or radiation, prolonged sitting down position) (Fig. 1). This difference was of high statistical significance (p 0.004). Also, 58 (52.3%), 41 (36.9%) and 55 (49.5%) were non-smokers, respectively. While, 50 (45.9%), 62 (56.8%) and 50 (45%) were smokers, respectively. The remainders were ex-smokers (Fig. 1). This difference was of non-statistical significance (p 0.102). Moreover, the majority of the patients in the 3 groups received hormonal treatment prior to micro-TESE (73 (66.7%), 50 (45%), 46 (42.3%), respectively), while the remainders did not receive hormonal treatment (Fig. 1). This difference was of high statistical significance (p 0.025).

Our study showed that the majority of the patients in the 3 groups had moderate testes volumes followed by, small, normal and finally atrophied testes (Fig. 2). These differences in the right and left testes volumes were of high statistical significance (p 0.001, 0.026, respectively). Furthermore, the main testicular histopathological pattern was sertoli cell only syndrome (SCO) followed by 1ry spermatocyte arrest, tubular sclerosis, 2ry spermatocyte arrest, spermatid arrest and finally hypospermatogenesis in the 3 groups (Fig. 2). This difference in testicular histopathology was of high statistical significance (p 0.007). Besides, the majority of the patients did not undergo varicocelectomy prior to micro-TESE (Fig. 2). This difference was also of statistical significance (p 0.034). Further, our study revealed a positive correlation between right testicular volume and favourable micro-TESE outcome in 1st timers who underwent micro-TESE (Table 2). This finding was explained by the presence of a significant difference between small testis in negative and positive cases (36.7% vs 13.7%). Thus, small-sized right testis tends to be associated with unfavourable micro-TESE outcome. Additionally, there was also a significant positive correlation between histopathology which was mainly SCO and favourable micro-TESE outcome in 1st timers micro-TESE (Table 2). On the contrary, our study did not reveal

Table 1 The age and the duration of infertility and the FSH level in the 3 groups and their correlations with micro-TESE outcome

	Final result						*P* value
	−ve			+ve			
	Median	1st quartile	3rd quartile	Median	1st quartile	3rd quartile	
1st timers TESE							
Age (years)	37.50	32.50	43.00	39.00	34.00	44.00	0.269
Duration of infertility (years)	7.00	3.50	12.00	8.00	3.00	12.00	0.917
FSH	16.70	12.05	23.00	14.90	6.90	17.70	*0.015*
2nd timers TESE							
Age (years)	38.00	36.00	46.00	35.50	30.50	41.00	*0.031*
Duration of infertility (years)	8.00	5.00	12.00	6.00	3.00	9.50	0.127
FSH	16.00	8.00	24.00	14.20	6.00	26.00	0.410
3rd timers TESE							
Age (years)	42.00	37.00	49.00	41.00	37.00	46.00	0.665
Duration of infertility (years)	11.00	6.00	18.00	10.00	8.00	14.00	0.858
FSH	21.70	14.10	30.00	21.25	15.20	42.75	0.772
	1st timers TESE		2nd timers TESE		3rd timers TESE		
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	
Age (years)	38.95	7.77	39.24	7.69	43.22	7.61	*< 0.001*
Duration of infertility (years)	8.14	5.50	8.77	6.31	12.33	7.33	*< 0.001*
FSH level (mIU/ml)	17.86	13.73	17.72	13.44	25.11	16.22	*< 0.001*

Significant correlations are in italics

N.B –ve, unfavourable micro-TESE outcome; +ve, favourable micro-TESE outcome

any association between varicocelectomy and hormonal treatment with favourable outcome in 1st timers micro-TESE (Table 2). Eventually, our study did not reveal any association between testicular volume, histopathology, hormonal treatment and varicocelectomy with favourable micro-TESE outcomes in 2nd and 3rd timers patients (Tables 3, 4).

Discussion

Remarkably, it is not uncommon that patients with previous testicular surgery and unsuccessful sperms retrieval could consider attempting another surgical treatment aimed at retrieving testicular sperms [14–17]. Interestingly, our study demonstrated a positive correlation between high FSH level and favourable micro-TESE outcome in 1st timers who underwent micro-TESE.

In addition, our study showed a significant correlation between the sperm retrieval rate (SRR) and the histopathological patterns, where the main histopathological patterns for 1st timers who underwent micr-TESE were SCO followed by 1ry spermatocyte arrest, tubular sclerosis, 2ry spermatocyte arrest, spermatid arrest and finally hypospermatogenesis 57 (51.8%), 34 (31%), 11 (10%), 5 (4.5%), 2 (1.8%), 1 (0.9%), respectively. This could be seen in the same line of the findings of Amer et al. [18] in our retrospective study. The sperm retrieval rate in the 1st timers who underwent micro-TESE was 80% (spermatid arrest) followed by 64.7% (1ry spermatocyte arrest), 50% (2ry spermatocyte arrest), 38.6% (SCO) and finally 18% (tubular sclerosis). SRR mentioned for 1st timer patients with SCO pattern (38.6%) in the present study was higher than that mentioned in the study conducted by Modarresi et al. (23.6%) and Amer et al. (33.3%), Okada et al. (33.9%) [11, 19, 20]. However, it was relatively close to that reported by Tsujimura et al. (39.1%) and Kalsi et al. (40%) but lower than that reported by Ramasamy et al. (41%) and Tsujimura et al. (42.9%) [2, 5, 6, 21]. Remarkably, this SSR for 1st timers with SCO was higher than that reported by Amer et al. who conducted a retrospective study as it was 29.6% [18].

Noteworthy, we obtained such higher SSR than our previous retrospective study by assigning senior andrologists with outstanding history of performing more than 150 micro-TESE operations during their professional career [22]. Moreover, we assigned at least 2 biologists were available in the operation theatres during the operation. Additionally, every kind of maturation arrest pattern in the present study reported SRR 55.1% that was higher than the most published results as they reported SRRs of 41.7%, 36%, and 44%, respectively [2, 6, 21]. Furthermore, our study

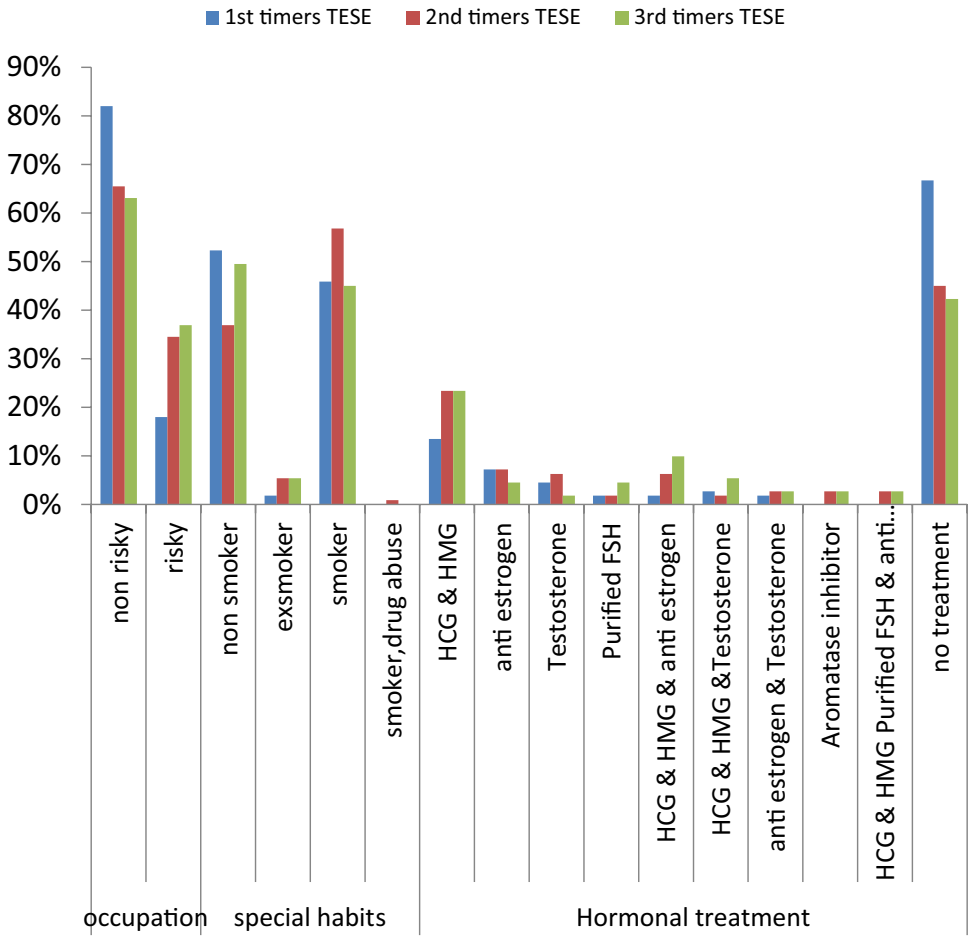

Fig. 1 The sociodemographic characteristics of the participants

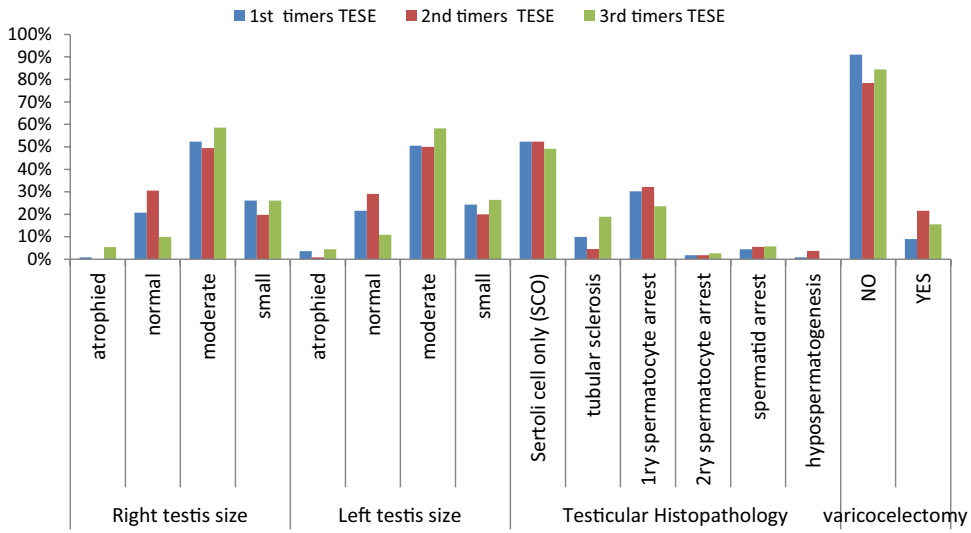

Fig. 2 The frequency of testicular volume and histopathology and varicocelectomy in the 3 groups

demonstrated a positive correlation between age in years and favourable micro-TESE outcome in 2nd timers patients. Similarly, Li et al. [23] demonstrated that age might be a predictive factor for successful retrieval of sperms. This finding can be explained by the fact that testicular degeneration in KF starts during foetal life and progresses during infancy and puberty into adulthood with subsequent depletion of spermatogonia that leads to abnormal seminiferous tubules [24, 25]. These seminiferous tubules either contain Sertoli cells only (SCO) or replaced by hyalinized material (tubular

Table 2 The relation between testicular volume and histopathology and effect of varicocelectomy and hormonal treatment with sperm retrieval rate in the 1st timers TESE group

1st timers TESE	Final result				*P* value
	−ve		+ve		
	Count	%	Count	%	
Rt testis volume					
Atrophied	0	0.0	1	2.0	*0.023*
Normal	11	18.3	12	23.5	
Moderate	27	45.0	30	60.8	
Small	22	36.7	7	13.7	
Lt testis volume					
Atrophied	3	5.0	1	2.0	0.173
Normal	11	18.3	13	25.5	
Moderate	27	45.0	28	56.9	
Small	19	31.7	8	15.7	
Histopathology					
Sertoli cell only syndrome (SCO)	35	60.0	22	43.1	*0.001*
Tubular sclerosis	9	15.0	2	3.9	
1ry spermatocyte arrest	12	20.0	22	43.1	
2ry spermatocyte arrest	1	1.7	1	2.0	
Spermatid arrest	1	1.7	4	7.8	
Hypospermatogenesis	1	1.7	0	0.0	
Hormonal treatment					
Yes	22	35	15	31.4	0.826
No	38	65.0	35	68.6	
Varicocelectomy					
No	54	90.0	47	92.2	0.751
Yes	6	10.0	4	7.8	

Significant correlations are in italics

Table 3 The relation between testicular volume and histopathology and effect of varicocelectomy and hormonal treatment with sperm retrieval rate in the 2nd timers TESE group

2nd timers TESE	Final result				*P* value
	−ve		+ve		
	Count	%	Count	%	
Rt testis size					
Atrophied	0	0.0	0	0.0	0.713
Normal	26	32.9	8	25.0	
Moderate	37	48.1	17	53.1	
Small	15	19.0	7	21.9	
Lt testis size					
Atrophied	0	0.0	1	3.1	0.487
Normal	24	30.8	8	25.0	
Moderate	39	50.0	16	50.0	
Small	15	19.2	7	21.9	
Histopathology					
Sertoli cell only syndrome (SCO)	44	56.4	13	40.6	0.108
Tubular sclerosis	4	5.1	1	3.1	
1ry spermatocyte arrest	23	29.5	11	34.3	
2ry spermatocyte arrest	1	1.3	3	9.3	
Spermatid arrest	3	3.8	3	9.3	
Hypospermatogenesis	3	3.8	1	3.1	
Hormonal treatment					
Yes	43	55.7	17	53.1	0.595
No	35	44.3	15	46.9	
Varicocelectomy					
No	64	82.3	22	68.8	0.117
Yes	14	17.7	10	31.2	

shadows) together with nodules of hyperplastic Leydig cells in the testicular interstitium [24, 26]. Otherwise, our study did not show any other correlations such as duration of infertility, FSH level, hormonal therapy, testicular volume, varicocelectomy and finally histopathology with favourable micro-TESE outcome in 2nd timer patients. However, it is noteworthy that the main histopathological patterns for 2nd timers who underwent micro-TESE were SCO followed by 1ry spermatocyte arrest, spermatid arrest, tubular sclerosis, 2ry spermatocyte arrest and finally hypospermatogenesis 57 (51.8%), 34 (31%), 6 (5.5%), 5 (4.5%), 4 (3.6%), 4 (3.6%), respectively.

The sperm retrieval rate in the 2nd timers who underwent micro-TESE was 75% (2ry spermatocyte arrest) followed by 50% (spermatid arrest), 32.4% (1ry spermatocyte arrest), 25% (hypospermatogenesis), 22.8% (SCO) and finally 20% (tubular sclerosis), respectively. Eventually, our study did not deliver out any positive correlation between age, duration of infertility, FSH level, hormonal therapy, testicular volume, varicocelectomy and finally histopathology with favourable micro-TESE outcome in 3rd timer patients. Moreover, it should be noted that the main histopathological patterns for 3rd timers who underwent micro-TESE were SCO followed by 1ry spermatocyte arrest, tubular sclerosis, spermatid arrest and finally 2ry spermatocyte arrest 55 (50%), 26 (23.6%), 18 (16.4%), 8 (7.3%), 3 (2.7%), respectively. Besides, the sperm retrieval rate in the 3rd timers who underwent micro-TESE was 37.5% (spermatid arrest) followed by 33.3% (2ry spermatocyte arrest), 25.4% (SCO), 23.1% (1ry spermatocyte arrest) and finally 5.6% (tubular sclerosis), respectively. However, it should be noted that in other reports, when looking only to NOA subpopulations with severe prognosis, similar low SRRs 30% and 26% were reported by Okada et al. and Franco et al. [20, 27], respectively. On the other hand, Ramasamy and Schlegel reported a sperm recovery rate of 45% using salvage micro-TESE (9/ 20), while it was 45.7% (21/ 46) and 46.5% (27/ 58) in Tsujimura and Kalsi observations [2, 6, 7]. Additionally, the

Table 4 The relation between testicular volume and histopathology and effect of varicocelectomy and hormonal treatment with sperm retrieval rate in the 3rd timers TESE group

3rd timers TESE	Final result				*P* value
	−ve		+ve		
	Count	%	Count	%	
Rt testis size					
Atrophied	4	4.4	2	9.5	0.073
Normal	7	7.8	4	19.0	
Moderate	51	57.8	13	61.9	
Small	27	30.0	2	9.5	
Lt testis size					
Atrophied	4	4.5	1	4.8	0.201
Normal	7	7.9	5	23.8	
Moderate	53	59.6	11	52.4	
Small	25	28.1	4	19.0	
Histopathology					
Sertoli cell only syndrome (SCO)	41	48.2	14	56	0.525
Tubular sclerosis	17	20	1	4	
1ry spermatocyte arrest	20	23.5	6	24	
2ry spermatocyte arrest	2	2.3	1	4	
Spermatid arrest	5	5.9	3	12	
Hypospermatogenesis	0	0.0	0	0.0	
Hormonal treatment					
Yes	56	61.1	8	42.9	0.731
No	34	38.9	12	57.1	
Varicocelectomy					
No	76	84.4	17	85.0	1
Yes	14	15.6	3	15.0	

sperm retrieval rate of salvage micro-TESE was 38.5% (20/52) in the study conducted by Xu et al. [28]. Remarkably, this work replicates the findings of our previous study that sperms can be retrieved despite high FSH level and preoperative histopathology is not essential in predicting SRR which can be explained by the fact that heterogeneous areas of histopathology may be found within the testes of patients with elevated FSH level [18, 29].

Additionally, age can be used as a prognostic indicator for NOA patients who undergo 2nd time micro-TESE. On the contrary, Ramasamy and Schlegel [7] concluded that there is no threshold of prior negative biopsies that precludes the success of sperm retrieval using micro-TESE. Furthermore, preoperative histopathology should be considered the most important factor in predicting SRRs in men who undergo salvage biopsy [30]. Eventually, the findings of this study were limited by their non-randomized and non-controlled natures. Furthermore, ICSI outcome for cases with successful sperm retrieval was not the scope of the present study.

Conclusion

Our prospective study demonstrates that sperms can be retrieved despite high FSH level and preoperative histopathology is not essential in predicting SRR in NOA patients. Further, age can be considered a good prognostic factor in patients who undergo salvage micro-TESE for the 2nd time.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the local ethical committee.

Informed consent Informed consent was obtained from all individual participants included in the study.

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