ARTICLE

Outcomes and predictive factors of successful salvage microdissection testicular sperm extraction (mTESE) after failed classic TESE: results from a multicenter cross-sectional study

Luca Boeri[1]✉, Carolina Bebi[1], Donato Dente[2], Ermanno Greco[3], Matteo Turetti[1], Marco Capece[4], Andrea Cocci[5], Gianmartin Cito[5], Mirko Preto[6], Edoardo Pescatori[7], Walter Ciampaglia[7], Fabrizio Ildefonso Scroppo[8], Marco Falcone[6], Carlo Ceruti[6], Franco Gadda[1], Giorgio Franco[9], Federico Dehò[8], Alessandro Palmieri[4], Luigi Rolle[6], Paolo Gontero[6], Francesco Montorsi[10,11], Emanuele Montanari[1] and Andrea Salonia[10,11]

Microdissection testicular sperm extraction (mTESE) has been proposed as a salvage treatment option for men with a previously failed classic TESE (cTESE), but data are scarce. We aimed to assess the outcome of and potential predictors of successful salvage mTESE in a cohort of men previously submitted to unfruitful cTESE. Data from 61 men who underwent mTESE after a failed cTESE between 01/2014 and 10/2020, at 6 tertiary-referral centres in Italy were analysed. All men were investigated with semen analyses, testicular ultrasound, hormonal and genetic blood testing. Pathological diagnosis from TESE was collected in every man. Descriptive statistics and logistic regression models were used to investigate potential predictors of positive sperm retrieval (SR+) after salvage mTESE. Baseline serum Follicle-Stimulating hormone (FSH) and total testosterone levels were 17.2 (8.6–30.1) mUI/mL and 4.7 (3.5-6.4) ng/mL, respectively. Sertoli-cell-only syndrome (SCOS), maturation arrest (MA) and hypospermatogenesis were found in 24 (39.3%), 21 (34.4%) and 16 (26.2%) men after cTESE, respectively. At mTESE, SR+ was found in 30 (49.2%) men. Patients with a diagnosis of hypospermatogenesis had a higher rate of SR+ (12/16 (75%)) compared to MA (12/21 (57.1%)) and SCOS (6/24 (25%)) patients at mTESE ($p < 0.01$). No clinical and laboratory differences were observed between SR+ and SR- patients at mTESE. There were no significant complications after mTESE. At multivariable logistic regression analysis, only hypospermatogenesis (OR 9.5; $p < 0.01$) was independently associated with SR+ at mTESE, after accounting for age and FSH.

In conclusion, salvage mTESE in NOA men with previous negative cTESE was safe and promoted SR+ in almost 50%. A baseline pathology of hypospermatogenesis at cTESE emerged as the only independent predictor of positive outcomes at salvage mTESE.

IJIR: Your Sexual Medicine Journal (2022) 34:795–799; https://doi.org/10.1038/s41443-021-00487-8

INTRODUCTION

Non obstructive azoospermia (NOA) is defined as the absence of sperm at the semen analysis after centrifugation, with usually a normal ejaculate volume [1]. It is a common clinical condition, accounting for approximately 1% of all men and 10% of all infertile men [2]. In this condition the severe deficit of spermatogenesis is often a consequence of primary testicular dysfunction or it can be related to a dysfunction of the hypothalamus-pituitary-gonadal (HPG) axis [3]. NOA men usually have small and isolated foci of residual spermatogenesis within the testes; therefore, surgical sperm retrieval and intracytoplasmic sperm injection (ICSI) are valuable treatment options to father a child.

Testicular sperm extraction (TESE) is the most common and effective procedures to retrieve sperm in NOA men, with a reported sperm retrieval rate of approximately 50% [4, 5]. Numerous predictive factors for positive sperm retrieval have been investigated, including clinical parameters, serum hormones, surgical approach and testicular histology, but no definitive predictors have been associated with successful retrieval [4–6]. Specifically, microdissection TESE (mTESE) has been associated with a 1.5 higher chance of retrieving sperm and lower rates of surgical complications compared to the conventional technique [4, 7]. However, the superiority of mTESE compared to conventional TESE (cTESE) is still a matter of debate. In fact, a recent meta-analysis showed that cTESE/mTESE, in NOA men, results in positive

[1]Department of Urology, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Milan, Italy. [2]Unit of Robotic & Mininvasive Surgery - Casa Di Cura Villa Igea, Ancona, Italy. [3]Centre for Reproductive Medicine, European Hospital, Rome, Italy. [4]Department of Neurosciences, Reproductive Sciences and Odontostomatology, University of Naples "Federico II", Naples, Italy. [5]Department of Urology and Andrology Surgery, University of Florence, Florence, Italy. [6]Division of Urology, A.O.U. Città della Salute e della Scienza di Torino - Presidio Molinette, Turin, Italy. [7]Reproductive Medicine Unit, GynePro Medical Centers, NextClinics International, Bologna, Italy. [8]Department of Urology and Andrology, Ospedale di Circolo e Fondazione Macchi, Varese, Italy. [9]Department Gynaecological-Obstetrical and Urological Sciences, Sapienza University of Rome, Rome, Italy. [10]Vita-Salute San Raffaele University, Milan, Italy. [11]Division of Experimental Oncology/Unit of Urology, URI; IRCCS Ospedale San Raffaele, Milan, Italy. ✉email: dr.lucaboeri@gmail.com

Received: 10 July 2021 Revised: 14 October 2021 Accepted: 21 October 2021
Published online: 6 November 2021

sperm retrieval of up to 50% of patients, with no differences between the two techniques [5]. Nonetheless, mTESE has been recently proposed as a treatment option for NOA men in whom cTESE has failed (namely, salvage mTESE) [8–10]. Previous Authors have investigated the clinical outcome of salvage mTESE (smTESE) procedures in NOA patients, showing promising results [8–10]. Of note, smTESE was associated with successful sperm retrieval in approximately 40% of men and an excellent safety profile even after a previous failed cTESE [8–10]. Nonetheless, published data come from single centre, small retrospective studies and potential predictors of successful sperm retrieval in these specific cohorts have not been definitively found.

These observations prompted us to conduct a multicenter study to investigate the rate of and potential predictors of sperm retrieval at smTESE in a cohort of NOA men with a previous failed cTESE in the real-life setting.

MATERIALS AND METHODS

Patient population

After institutional review board approval, we retrospectively reviewed data from 64 consecutive NOA patients who had a cTESE where no sperm were found and subsequently underwent smTESE at six academic centres between January 2014 and October 2020. NOA was defined as the absence of sperm in two consecutive semen analyses after centrifugation of the samples [1]. Obstructive causes were excluded with a complete clinical and diagnostic work-up. All patients were assessed with a thorough sexual and medical history. The Charlson Comorbidity Index (CCI) was used to score health-significant comorbidities, categorized as 0 vs. ≥ 1 [11]. Measured body mass index (BMI) was obtained for each participant. Testis volume was evaluated with ultrasound scanning and varicocele was clinically assessed in every patient [3, 12].

Venous blood samples were drawn from each patient between 7 AM–11 AM after an overnight fast. Circulating serum hormone levels, including follicle-stimulating hormone (FSH), luteinizing hormone (LH) and total testosterone (tT), were measured in every patient. Similarly, karyotype analysis and genetic testing were performed in every patient (i.e., Y-chromosome microdeletions and cystic fibrosis mutations) [13].

Likewise, testicular pathology from the cTESE procedure was collected in each patient.

None of the patients had received hormonal treatment (e.g. human chorionic gonadotropin, oestrogen receptor modulators or aromatase inhibitors) before mTESE to improve sperm retrieval rate (SRR).

Surgical techniques

smTESE have been performed at least 6 months following the primary cTESE procedure [14, 15]. Informed consent was obtained after a thorough explanation of published data and the invasiveness of the surgical technique. In brief, smTESE was performed under general anaesthesia with the patient in the supine position. Through a mid-line scrotal incision on the median raphe of the scrotum, the skin, dartos muscle, and tunica vaginalis were opened to expose the tunica albuginea. A transverse equatorial incision was made on each testis and each side of the tunical incision was gently pulled apart with the aid of two surgical haemostats [16]. The testicular parenchyma was examined under at least 25x magnification using a surgical microscope. Multiple testicular specimens were excised from opaque, enlarged tubules with microforceps and collected in medium (HEPES/human tubal fluid medium with 5% albumin). All samples were immediately evaluated by an embryologist using a 200x magnification microscope in order to investigate the presence of spermatozoa. If spermatozoa were not detected in the first samples, additional samples were obtained from the same testicle and eventually from the contralateral testis. At the same time of testicular intervention, a small tissue specimen was sent for final histopathological examination. All procedures were performed skin-to-skin by surgeons with at least five years of experience in mTESE. Positive sperm retrieval (SR+) was defined as finding at least one spermatozoa that could be preserved or used for ICSI [4].

Overall, two (3.1%) patients were excluded due to the lack of histological reports at cTESE and one (1.5%) patient with a karyotype result of Klinefelter Syndrome (47, XXY). A convenient sample of 61 (95.3%) NOA men who underwent smTESE was considered for the final analysis.

Data collection followed the principles outlined in the Declaration of Helsinki. All patients signed an informed consent agreeing to share their own anonymous information for future studies. The study was approved by the IRCCS Foundation Ca' Granda–Maggiore Policlinico Hospital Ethical Committee (Prot. 25508).

Statistical analyses

Distribution of data was tested with the Shapiro–Wilk test. Data are presented as medians (interquartile range; IQR) or frequencies (proportions). The Mann-Whitney test and Chi Square test were used to test the association between clinical characteristics and laboratory values between patients with SR+ and those with negative SR (SR-). Univariable and multivariable logistic regression models were used to identify variables associated with SR+ in the whole cohort. Statistical analyses were performed using SPSS v.26 (IBM Corp., Armonk, NY, USA). All tests were two sided, and the statistical significance level was determined at $P < 0.05$.

RESULTS

Table 1 details clinical characteristics of 61 NOA men submitted to smTESE. At cTESE, pathology reports showed Sertoli cell-only syndrome (SCOS), maturation arrest and hypospermatogenesis in 24 (39.3%), 21 (34.4%) and 16 (26.2%) patients, respectively. At smTESE, median (IQR) operative time was 70 (55-106) minutes. Patient's age and testicular volume at salvage surgery were 35 (31–38) years and 10 [6–15] ml, respectively. Above all, 16 (26.3%) participants had CCI ≥ 1. Current smoking status and alcohol consumption was reported by 23 (37.7%) and 28 (45.9%) men, respectively. Baseline serum FSH and total testosterone levels were 17.2 (8.6–30.1) mUI/mL and 4.7 (3.5–6.4) ng/mL, respectively. Overall, positive SRR was 49.2% (30 out of 61 men). Only 1 (1.6%) patient had complications after smTESE (scrotal haematoma).

Table 2 details patients' characteristics according to SR outcomes. SR+ and SR− patients did not differ in terms of clinical, hormonal and procedural parameters (all $p > 0.05$). Patients with a diagnosis of hypospermatogenesis at cTESE had a higher rate of SR+ [12/16 (75%)] than those with maturation arrest [12/21 (57.1%)] and SCOS [6/24 (25%)] at smTESE, respectively ($p < 0.01$).

At univariable logistic regression analyses, hypospermatogenesis (OR 9.1, $p < 0.01$) and maturation arrest (OR 4.2, $p = 0.03$) at cTESE were associated with SR+ (Table 3). At multivariable logistic regression analysis hypospermatogenesis (OR 9.5, $p < 0.01$) was the only independent predictor of positive SR after accounting for age and FSH (Table 3).

DISCUSSION

smTESE has been proposed as a promising technique for sperm retrieval in men with a previous failed cTESE. Data from the literature reported that smTESE was effective in approximately 40% of men, with an excellent safety profile even after a previous negative procedure [8–10]. However, the efficacy of smTESE has been investigated only in single centre studies and potential predictors of successful SR in this specific cohort have not been clearly identified.

This gap in the literature prompted us to conduct a multicenter, national, retrospective analysis with the specific aim of investigating the outcome of and the safety profile of smTESE, also identifying potential predictors of SR+. Overall, we found that spermatozoa could be retrieved with smTESE in almost half of patients (49.2%) who had a previous negative cTESE. In this context, a histological diagnosis of hypospermatogenesis emerged as the only independent predictor of SR+ at smTESE, after accounting for clinical and hormonal characteristics. The option to offer a second chance of sperm recovery and identify the most suitable candidate for smTESE is of major clinical relevance, since unsuccessful SR at the first TESE results in sexual impairment and negative emotional effects for the couple [17, 18].

Table 1. Characteristics and descriptive statistics of the whole cohort (No.=61).

Age (years)	
Median (IQR)	35.0 (31–38)
Range	18–55
BMI (Kg/m^2)	
Median (IQR)	25.1 (22.8–27.2)
Range	18.5–37.0
CCI [No. (%)]	
CCI 0	45 (73.7)
CCI ≥ 1	16 (26.3)
Testicular volume (ml)	
Median (IQR)	10.0 (6–15)
Range	3–25
Current smokers [No. (%)]	23 (37.7)
Alcohol consumers [No. (%)]	28 (45.9)
Varicocele [No. (%)]	26 (42.6)
FSH (mUI/mL)	
Median (IQR)	17.2 (8.6–30.1)
Range	3.5–50.0
LH (mUI/mL)	
Median (IQR)	9.7 (7.1–12.4)
Range	3.2–24.0
tT (ng/mL)	
Median (IQR)	4.7 (3.5–6.4)
Range	1.1–12.8
Histologic reports [No. (%)]	
Maturation arrest	21 (34.4)
Sertoli cell-only syndrome	24 (39.3)
Hypospermatogenesis	16 (26.2)
Operative time (min)	
Median (IQR)	70.0 (55–106)
Range	32–130
Positive SR+ [No. (%)]	30 (49.2)
Postop. complications [No. (%)]	1 (1.6)

BMI body mass index, *CCI* Charlson Comorbidity Index, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *tT* total testosterone, *SR+* positive sperm retrieval, *smTESE* salvage microdissection testicular sperm extraction.

Table 2. Characteristics and descriptive statistics of patients according to SRR (No.=61).

	SR+ (*N* = 30; 49.2%)	**SR− (*N* = 31; 50.8%)**	***p* value***
Age (years)			0.6
Median (IQR)	34.0 (30–39)	36.0 (32–39)	
Range	18–56	27–47	
BMI [Kg/m^2]			0.8
Median (IQR)	25.1 (20.6–26.2)	25.0 (22.9–26.5)	
Range	20.5–37.0	18.5–35.8	
CCI ≥ 1 [No. (%)]	4 (12.9)	12 (38.7)	0.1
Testicular volume (ml)			0.1
Median (IQR)	11.0 (8.0–16.0)	7.8 (5.5–15.0)	
Range	3.0–24.0	3.0–20.0	
Current smokers [No. (%)]	9 (30.0)	14 (45.1)	0.5
Alcohol consumers [No. (%)]	12 (40.0)	16 (51.6)	0.6
Varicocele [No. (%)]	13 (43.3)	13 (41.9)	0.8
FSH (mUI/mL)			0.5
Median (IQR)	14.7 (8.5–25.0)	18.0 (8.7–38.5)	
Range	5.1–43.0	3.5–50.0	
LH (mUI/mL)			0.9
Median (IQR)	9.7 (5.5–15.7)	9.7 (8.2–12.0)	
Range	3.2–24.0	3.9–21.3	
tT (ng/mL)			0.7
Median (IQR)	4.9 (3.8–6.7)	4.7 (3.2–6.2)	
Range	1.1–9.8	1.4–12.8	
Operative time (min)			0.3
Median (IQR)	72.5 (59–108)	56.0 (47–105)	
Range	50–130	22–120	
Bilateral surgery [No. (%)]	18 (60.0)	18 (58.1)	0.9
Histologic reports at cTESE [No. (%)]			<0.01
Sertoli cell-only syndrome	6 (20.0)	18 (58.1)	
Maturation arrest	12 (40.0)	9 (29.0)	
Hypospermatogenesis	12 (40.0)	4 (12.9)	

BMI body mass index, *CCI* Charlson Comorbidity Index, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *tT* total testosterone, *cTESE* conventional testicular sperm extraction, *SRR* Sperm retrieval rate.
**p* value according to the Mann–Whitney test and Chi Square test, as indicated.

On the one hand, current findings confirm previous observations. Indeed, Tsujimura et al [8]. were among the first that investigated outcomes of smTESE in a cohort of 46 NOA men. Authors found that SRR of smTESE was similar to that of primary mTESE (45.7% vs. 44.0%), but no predictors of SR+ were identified. Subsequently, Kalsi et al [9]. retrospectively analysed data from 58 NOA men who underwent smTESE between 2008 and 2013. All patients were previously submitted to single or multiple cTESE or testicular sperm aspiration (TESA) where no sperm was found. Conversely, positive SRR at smTESE was 46.5% in this cohort, and men with SR+ showed higher serum testosterone levels and a higher frequency of hypospermatogenesis histology at first surgery than those with negative recovery [9].

Previous reports have questioned whether or not preoperative histopathology correlates with SRRs [6, 19, 20]. The presence of hypospermatogenesis at testicular biopsy showed good accuracy in predicting SR+ at mTESE as compared with either maturation arrest pattern or SCOS [19, 20]. This was also confirmed in the previous smTESE series [9, 10]. Kalsi et al. found that SR+ was more frequent in patients with hypospermatogenesis (75%) than those with maturation arrest (36%) and SCOS (40%) [9]. Similar results were reported by Xu et al. [10]. Thereof, our results corroborate the importance of preoperative histology in terms of smTESE outcomes prediction. Indeed, we found that patients with a diagnosis of hypospermatogenesis at cTESE had a higher rate of SR+ (75%) than those with maturation arrest (57.1%) and SCOS

Table 3. Univariable and multivariable logistic regression models predicting SR+ in the whole cohort of patients (No.=61).

	OR	*p* value	95% CI	OR	*p* value	95% CI
Univariable analysis	**Multivariable analysis**					
Age	0.98	0.62	0.91; 1.06	0.93	0.31	0.82; 1.09
CCI ≥ 1	0.87	0.76	0.23; 2.45			
Current smoking status	0.63	0.58	0.13; 3.09			
Alcohol consumption	0.66	0.61	0.14; 3.04			
Testis volume	1.11	0.26	0.92; 1.46			
Varicocele	0.94	0.34	0.65; 2.03			
FSH	0.98	0.33	0.95; 1.08	0.95	0.35	0.80; 1.22
Total testosterone	1.06	0.24	0.96; 2.15			
Operative time	1.01	0.38	0.98; 1.08			
Testicular histology at cTESE						
Sertoli cell-only syndrome	Ref		Ref	Ref		Ref
Hypospermatogenesis	9.1	<0.01	2.08; 15.78	9.5	<0.01	2.73; 19.6
Maturation arrest	4.2	0.03	1.12; 13.59	3.5	0.07	0.84; 12.8

SR Sperm retrieval, *OR* odds ratio, *95% CI* 95% confidence interval, *CCI* Charlson Comorbidity Index, *FSH* follicle-stimulating hormone, *cTESE* conventional testicular sperm extraction.

(25%) at smTESE; moreover, testicular histology emerged as the only predictor of SR+ after adjusting for clinical and hormonal characteristics.

Despite the importance of histology result on TESE outcome, a diagnostic testicular biopsy is not recommended in clinical practice due to the additional cost, repetitive surgical procedures, and the invasive nature of the procedure that increase the risk of complications [3]. In this study, all centres performed routine testicular histopathology investigations at first cTESE, which enables us to give reasonable suggestions of SR+ probability at smTESE. Moreover, it should be mentioned that even patients with extremes of spermatogenic failure (e.g., SCOS) may harbour focal areas of spermatogenesis [9, 21]. Indeed, we observed that one out of four men with SCOS had SR+ at smTESE, which was inferior to hypospermatogenesis or maturation arrest, but this result suggests that a non-negligible number of patients will have sperm found despite an adverse histopathological diagnosis.

Serum hormones and gonadotropins have been investigated as potential predictors of sperm retrieval at smTESE. Yücel et al. [18] analysed data from 49 NOA men who underwent smTESE after a previously failed mTESE and showed that men with SR+ had higher FSH values than those with negative outcomes. Moreover, FSH was the only predictor of positive SR at smTESE after accounting for age, LH, and Johnsen's score [18]. Our study, in line with previous reports [9, 10], failed to find any association between FSH values and smTESE outcome. FSH acts by binding to its receptors on the Sertoli cells, which are important for spermatogenesis in the testis. In clinical practice, FSH level is thought to be inversely correlated with the impairment of the overall spermatogenesis and inversely related to the total number of germ cells present. However, the value of mTESE is the ability of identifying the most advanced testicular pattern, not necessarily the predominant pattern of spermatogenesis. As a consequence, FSH might not be a good predictor for the identification of isolated areas of mature spermatogenesis.

Similarly, a preserved function of the Leydig compartment was found to be related to smTESE outcomes. Previous Authors showed that higher baseline serum testosterone levels were associated with SR+ at smTESE [9, 10], but this was not confirmed by subsequent reports [8, 22]. Our study confirmed the lack of association between preoperative testosterone levels and smTESE outcomes. Overall, despite high levels of FSH and LH would tend to indicate a global failure of sperm production, topographical variation of testicular pathology can occur irrespective of testicular function and single foci of spermatogenesis may also be present despite high gonadotropins levels. The role of mTESE is crucial in these patients, since the possibility to analyse thoroughly and systematically the testes can allow to find isolated foci of preserved spermatogenesis even in patients with global failure of sperm production.

Consistent with all previous reports on the same topic, we did not record any significant complications after smTESE, with only one patient who had scrotal hematoma after the procedure treated conservatively. Therefore, we could confirm that smTESE is a safe procedure even in patients who had a previous testicular surgery.

The clinical strength of our study is several-fold. First, we report findings from the largest multiceter study of smTESE in the real-life setting. Second, we identified hypospermatogenesis histology as a potential predictor of SR+ in this specific cohort; on the contrary, we showed that clinical, hormonal and procedural factors are unable to predict the SR+ in smTESE. In this context, we believe that NOA patients should be carefully counselled regarding their chance of retrieving spermatozoa after a previous failed cTESE.

Further strength of present study is that we have comprehensively investigated a relatively large homogenous group of patients with a detailed clinical, hormonal evaluation and an accurate assessment of the first cTESE. On the contrary, other authors have included: (i) patients after TESA [9], which is no longer recommended for NOA men [3], raising the possibility of the inclusion of men with obstructive azoospermia and (ii) men with Klinefelter syndrome [8, 9, 22], whose rate of SR is debated in the recent literature [23, 24].

Our study is not devoid of limitations. First, this study was a multicenter-based cross-sectional investigation, thus raising the possibility of selection biases. However, this could even be a significant strength; indeed, the fact that the study includes surgical cases from several centres strengthens the feasibility data of the surgical method and the multivariable predictivity of the pathology parameter, not being burdened by the potential bias (either negative or positive) of a single surgeon and a single embryologist. Second, despite the fact that we analyzed a relatively large, homogeneous, same-race cohort of NOA men submitted to smTESE, our study could be underpowered to infer association between predictors and SR outcomes; thereof, larger studies are needed to externally validate our findings. Third, because not recommended by current scientific guidelines [3], no patients have been treated with gonadotropins, clomiphene or human chorionic gonadotropin before smTESE; therefore, we could not depict the impact of hormonal therapy toward SRR in

this cohort. Fourth, we did not perform the long-term follow-up of our cohort, therefore we could not investigate the long-term sequalae of repeated TESE in terms of hormone parameters. Lastly, as noted for the majority of TESE investigations [25], we lacked data on reproductive outcome for this cohort, which might represent a stronger clinical endpoint compared to SRR.

CONCLUSIONS

The results of this cross-sectional, multicenter, real-life study revealed that in approximately half of patients (49.2%) who had a previous negative cTESE, spermatozoa could be retrieved by using mTESE. smTESE can be safely performed with no major early post-surgical complications.

Of clinical note, the histological diagnosis of hypospermatogenesis was the only independent predictor of SR+ at smTESE, after accounting for clinical and hormonal characteristics.

Overall, these observations pointed out the importance of accurate counselling for NOA men with SR- at first TESE in terms of subsequent chance of sperm recovery by microdissection procedures. Given the paucity of data in the literature on this topic, further larger cohort studies are needed to corroborate our results.

REFERENCES

1. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HWG, Behre HM, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update. 2010;16:231–45.
2. Cocuzza M, Alvarenga C, Pagani R. The epidemiology and etiology of azoospermia. Clin (Sao Paulo). 2013;68:15–26. Suppl 1.
3. Minhas S, Bettocchi C, Boeri L, Capogrosso P, Carvalho J, Cilesiz NC, et al. European Association of Urology Guidelines on Male Sexual and Reproductive Health: 2021 update on male infertility. Eur Urol. 2021;S0302-2838:01982–5.
4. Bernie AM, Mata DA, Ramasamy R, Schlegel PN. Comparison of microdissection testicular sperm extraction, conventional testicular sperm extraction, and testicular sperm aspiration for nonobstructive azoospermia: a systematic review and meta-analysis. Fertil Steril. 2015;104:1099–1103.e1-3.
5. Corona G, Minhas S, Giwercman A, Bettocchi C, Dinkelman-Smit M, Dohle G, et al. Sperm recovery and ICSI outcomes in men with non-obstructive azoospermia: a systematic review and meta-analysis. Hum Reprod Update. 2019;25:733–57.
6. Maglia E, Boeri L, Fontana M, Gallioli A, De Lorenzis E, Palmisano F, et al. Clinical comparison between conventional and microdissection testicular sperm extraction for non-obstructive azoospermia: Understanding which treatment works for which patient. Arch Ital Urol Androl. 2018;90:130–5.
7. Deruyver Y, Vanderschueren D, Van der Aa F. Outcome of microdissection TESE compared with conventional TESE in non-obstructive azoospermia: a systematic review. Andrology. 2014;2:20–4.
8. Tsujimura A, Miyagawa Y, Takao T, Takada S, Koga M, Takeyama M, et al. Salvage microdissection testicular sperm extraction after failed conventional testicular sperm extraction in patients with nonobstructive azoospermia. J Urol. 2006;175:1446–9. discussion 1449.
9. Kalsi JS, Shah P, Thum Y, Muneer A, Ralph DJ, Minhas S. Salvage micro-dissection testicular sperm extraction; outcome in men with non-obstructive azoospermia with previous failed sperm retrievals. BJU Int. 2015;116:460–5.
10. Xu T, Peng L, Lin X, Li J, Xu W Predictors for successful sperm retrieval of salvage microdissection testicular sperm extraction (TESE) following failed TESE in non-obstructive azoospermia patients. Andrologia. 2017;49.
11. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40:373–83.
12. Boeri L, Capogrosso P, Ventimiglia E, Cazzaniga W, Pozzi E, Belladelli F, et al. Testicular volume in infertile versus fertile white-European men: a case-control investigation in the real-life setting. Asian J Androl. 2021;23:501–9.
13. Ventimiglia E, Capogrosso P, Boeri L, Pederzoli F, Cazzaniga W, Scano R, et al. When to perform karyotype analysis in infertile men? validation of the European Association of urology guidelines with the proposal of a new predictive model. Eur Urol. 2016;70:920–3.
14. Amer M, Haggar SE, Moustafa T, Abd El-Naser T, Zohdy W. Testicular sperm extraction: impact of testicular histology on outcome, number of biopsies to be performed and optimal time for repetition. Hum Reprod. 1999;14:3030–4.
15. Schlegel PN, Su LM. Physiological consequences of testicular sperm extraction. Hum Reprod. 1997;12:1688–92.
16. Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. Hum Reprod. 1999;14:131–5.
17. Akbal C, Mangir N, Tavukçu HH, Ozgür O, Simşek F. Effect of testicular sperm extraction outcome on sexual function in patients with male factor infertility. Urology. 2010;75:598–601.
18. Yücel C, Budak S, Keskin MZ, Kisa E, Kozacioglu Z. Predictive factors of successful salvage microdissection testicular sperm extraction (mTESE) after failed mTESE in patients with non-obstructive azoospermia: Long-term experience at a single institute. Arch Ital Urol Androl. 2018;90:136–40.
19. 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.
20. Caroppo E, Colpi EM, Gazzano G, Vaccalluzzo L, Scroppo FI, D'Amato G, et al. Testicular histology may predict the successful sperm retrieval in patients with non-obstructive azoospermia undergoing conventional TESE: a diagnostic accuracy study. J Assist Reprod Genet. 2017;34:149–54.
21. Kalsi J, Thum M-Y, Muneer A, Abdullah H, Minhas S. In the era of micro-dissection sperm retrieval (m-TESE) is an isolated testicular biopsy necessary in the management of men with non-obstructive azoospermia? BJU Int. 2012;109:418–24.
22. Özman O, Tosun S, Bayazıt N, Cengiz S, Bakırcıoğlu ME. Efficacy of the second micro-testicular sperm extraction after failed first micro-testicular sperm extraction in men with nonobstructive azoospermia. Fertil Steril. 2021;115:915–21.
23. Boeri L, Palmisano F, Preto M, Sibona M, Capogrosso P, Franceschelli A, et al. Sperm retrieval rates in non-mosaic Klinefelter patients undergoing testicular sperm extraction: What expectations do we have in the real-life setting? Andrology. 2020;8:680–7.
24. Pozzi E, Boeri L, Capogrosso P, Palmisano F, Preto M, Sibona M, et al. Rates of hypogonadism forms in Klinefelter patients undergoing testicular sperm extraction: A multicenter cross-sectional study. Andrology. 2020;8:1705–11.
25. Ernandez J, Berk B, Han T, Abou Ghayda R, Kathrins M. Evaluating the quality of reported outcomes for microsurgical TESE in men with non-obstructive azoospermia: A methodological analysis. Andrology. 2021;9:1108–18.

AUTHOR CONTRIBUTIONS

LB was responsible for designing the protocol, collecting data, interpreting results, performing statistical analysis and drafting the manuscript. CB was responsible for collecting data, interpreting results. DD was responsible for collecting data, interpreting results. EG was responsible for collecting data, interpreting results. MT was responsible for collecting data, interpreting results. MC was responsible for collecting data, interpreting results. AC was responsible for collecting data, interpreting results. GC was responsible for collecting data, interpreting results. MP was responsible for collecting data, interpreting results. EP was responsible for collecting data, interpreting results. WC was responsible for collecting data, interpreting results. FS was responsible for collecting data, interpreting results. MF was responsible for collecting data, interpreting results. CC was responsible for collecting data, interpreting results. FG was responsible for collecting data, interpreting results. GF was responsible for collecting data, interpreting results. FD was responsible for collecting data, interpreting results, supervising the study. AP was responsible for collecting data, interpreting results, supervising the study. LR was responsible for collecting data, interpreting results, supervising the study. PG was responsible for collecting data, interpreting results, supervising the study. FM was responsible for collecting data, interpreting results, supervising the study. EM was responsible for collecting data, interpreting results, supervising the study. AS was responsible for collecting data, interpreting results, supervising the study.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Luca Boeri.