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ORIGINAL RESEARCH

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Shear wave elastography for predicting the likelihood of sperm retrieval in microscopic testicular sperm extraction

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Abstract

Background: The gold standard procedure for retrieving mature sperm cells from the foci of spermatogenesis in the testicles of patients with nonobstructive azoospermia (NOA) is called microscopic testicular sperm extraction (micro-TESE). Shear wave elastography (SWE) is an imaging technique that measures the modulus of elasticity, which represents the stiffness of tissues. We investigated the effectiveness of testicular elastography in predicting the probability of sperm retrieval during micro-TESE in patients with azoospermia. **Methods**: We prospectively included 84 patients diagnosed with NOA who were scheduled to undergo micro-TESE. Prior to micro-TESE, testicular elastography was performed on all patients. Six SWE measurements were recorded for each patient, representing the right upper, right middle, right lower, left upper, left middle and left lower regions. After SWE measurement, the patients underwent a micro-TESE procedure performed by the same surgeon. The patients were divided into TESE-positive and TESE-negative groups. By contrast, statistically significant differences were observed between the Klinefelter Syndrome (KS) and non-KS groups in other clinical and hormonal parameters, including Follicle-Stimulating Hormone (FSH) (p = 0.003), Luteinizing Hormone (LH) (p = 0.001), Total Testosterone (TT) (p = 0.047), and estradiol (p = 0.041), $p < 0.05$ and TESE-negative. The hormone levels, SWE values, and genetics of the two groups of patients were compared. **Results**: Following the micro-TESE procedures, sperm retrieval was successful in 45 patients (53.57%) and unsuccessful in 39 patients (46.43%). There were no significant differences between the SWE values of the TESE-positive and TESE-negative groups. Significantly higher SWE values were found in the Klinefelter syndrome group than in patients with normal genetics. **Conclusions**: Although testicular elastography is a noninvasive and easily applicable imaging method, it cannot be used to predict sperm retrieval in patients with NOA. **Clinical Trial Registration**: The Unique Identifier NCT06524258 and the URL is https://clinicaltrials.gov/study/NCT06524258.

Keywords

Shear wave elastography; Microscopic testicular sperm extraction; Azoospermia; Male infertility; Testis

1. Introduction

Infertility is a condition wherein spontaneous pregnancy is not achieved despite couples being sexually active and performing unprotected intercourse for at least a year [1]. Approximately 15% of married couples experience infertility within the first year of marriage [2]. Azoospermia has a prevalence of 1% in the general male population and 10–15% in the infertile population [3]. It is categorized into obstructive azoospermia (OA) and nonobstructive azoospermia (NOA) [4, 5]. The procedure for retrieving mature sperm cells from the foci of spermatogenesis in the testicles of patients with NOA is called testicular sperm extraction (TESE). The sperm retrieval rate (SRR) of the TESE procedure is approximately 20–45%; however, this increases to about 60% when the microscopic TESE (micro-TESE) technique is used [6]. Therefore, micro-TESE is considered the gold standard method for surgical sperm retrieval [7].

Testicular elastography allows for the acquisition of new data that can be used in the structural and functional evaluation of testicular tissue [8, 9]. New ultrasonography techniques, such as strain elastography (SE) and shear wave elastography (SWE), have recently been developed for imaging low-speed blood flow and structural changes in tissue [10]. SWE is an imaging technique that can measure the modulus of elasticity,

which represents the stiffness of tissues [11]. In SWE, a pulse applied to the tissue induces the formation of transverse waves arranged perpendicular to the direction of the ultrasound beam [12]. This study investigates the effectiveness of testicular elastography in predicting the likelihood of sperm retrieval following a micro-TESE in patients with azoospermia.

2. Materials and methods

2.1 Patients

Before commencing our study, we obtained approval from the local ethics committee. The study focused on patients who visited our urology outpatient clinic for infertility between January 2020 and March 2021. Eighty-four patients diagnosed with NOA were prospectively enrolled in the study. Patients with OA, hypogonadotropic hypogonadism, a history of orchiopexy, previous TESE, varicoceles or previous varicocele surgery, Y deletion, or a history of chemotherapy or radiotherapy were excluded from the study.

Physical examination included assessing secondary sex characteristics, testicular volumes and consistency, palpation of the vas deferens, checking for epididymis dilatation, and examining for the presence of varicoceles and other systemic findings. Azoospermia was diagnosed after performing at least two semen analyses, adhering to the criteria outlined in the sixth edition of the World Health Organization (WHO) manual for human semen analysis. Preoperative parameters, such as follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, prolactin, and estradiol levels, were analyzed. Additionally, all patients underwent karyotype analysis and Y microdeletion analysis. Testicular volumes were measured using Prader orchidometry and confirmed with scrotal ultrasonography. None of the patients received medical treatment before the micro-TESE procedure.

2.2 Elastography procedure

For patients scheduled for micro-TESE, elastography measurements were conducted in the supine position using an Acuson S2000 device (Siemens, Erlangen, BY, Germany) in the ultrasonography room before the operation. These measurements were performed by the same radiologist in the radiology department. A total of six points of SWE measurements were recorded in each patient, including upper right, middle right, lower right, upper left, middle left, and lower left poles (Fig. 1).

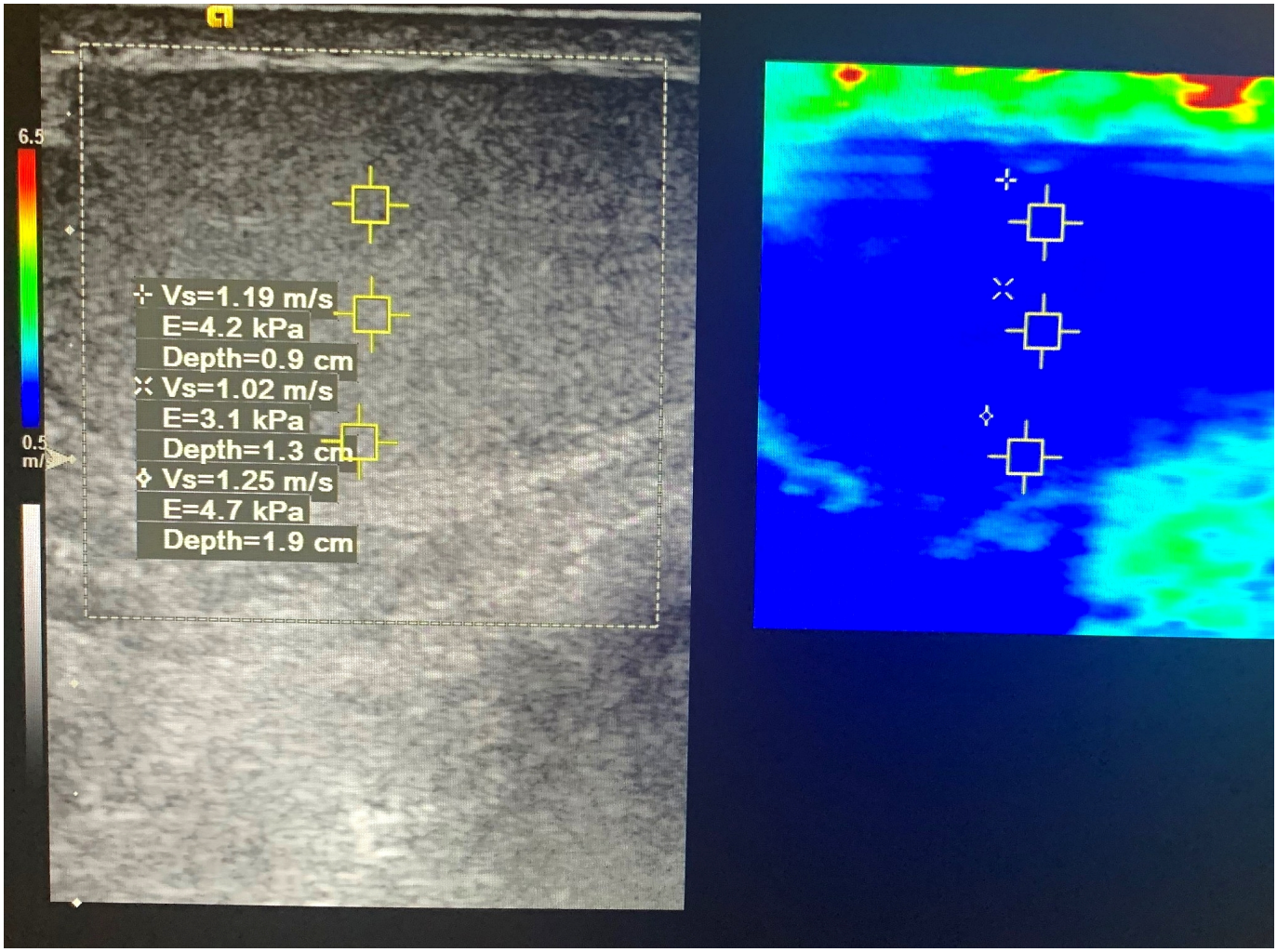

FIGURE 1. Elastography image of a TESE-positive patient with azoospermia. The image of three points of shear wave elastography measurements from each testis, including the upper, middle and lower poles.

2.3 TESE procedure

All patients underwent the micro-TESE procedure, which was conducted by the same surgeon. The procedure was first applied to the testis with a better volume and consistency (Fig. 2). Samples of large and bright tubules were extracted using microforceps under a microscope, utilizing a magnification range of 20–25×. The tissues obtained were subsequently assessed by the same embryologist who was present in the operating room. The embryologist provides biopsy results indicating the presence or absence of spermatozoa. If five or more mature spermatozoa are observed within the testicular tissues, the procedure is terminated (Fig. 3). However, if fewer than five spermatozoa are identified in the tissues, the procedure is repeated on the contralateral testicle to ensure a comprehensive examination and thorough exploration. Tissues containing a satisfactory quantity of sperm were processed and preserved in the incubator until the intracytoplasmic sperm injection (ICSI) procedure was performed, which was planned following the sperm cryopreservation of the patients whose sperm could be retrieved. When sperm could not be retrieved, the patients' testicular tissue was placed in Bouin's solution and delivered to the pathology laboratory for histopathological examination.

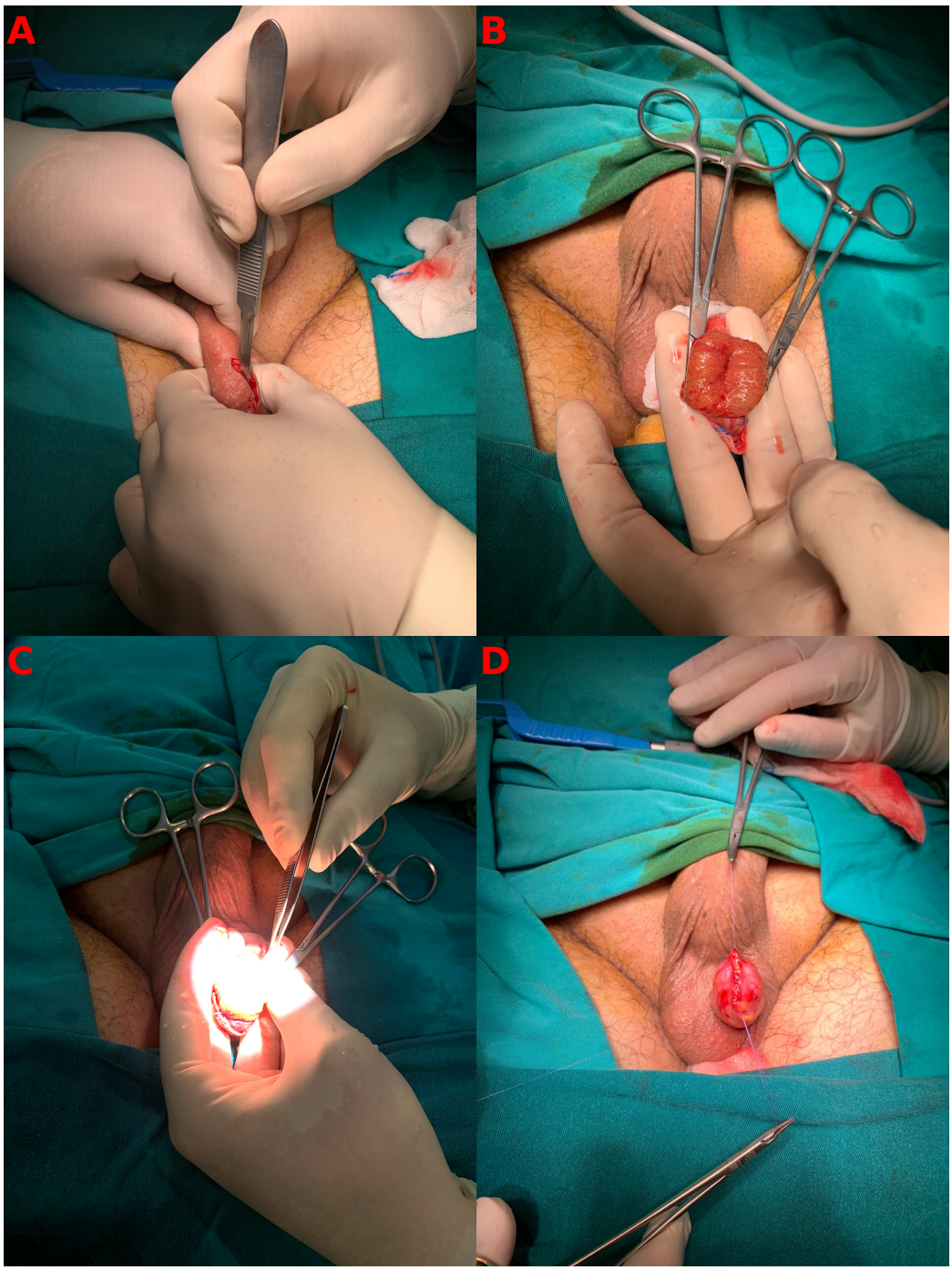

FIGURE 2. Images of a micro-TESE surgery. (A) The testicle is accessed via a vertical incision from the scrotal raphe. (B) The tunica albuginea is then incised, and the testicle is divided in two. (C) The seminiferous tubules are accessed and dissected with the guidance of a microscope. (D) Following the procedure, the layers are anatomically closed.

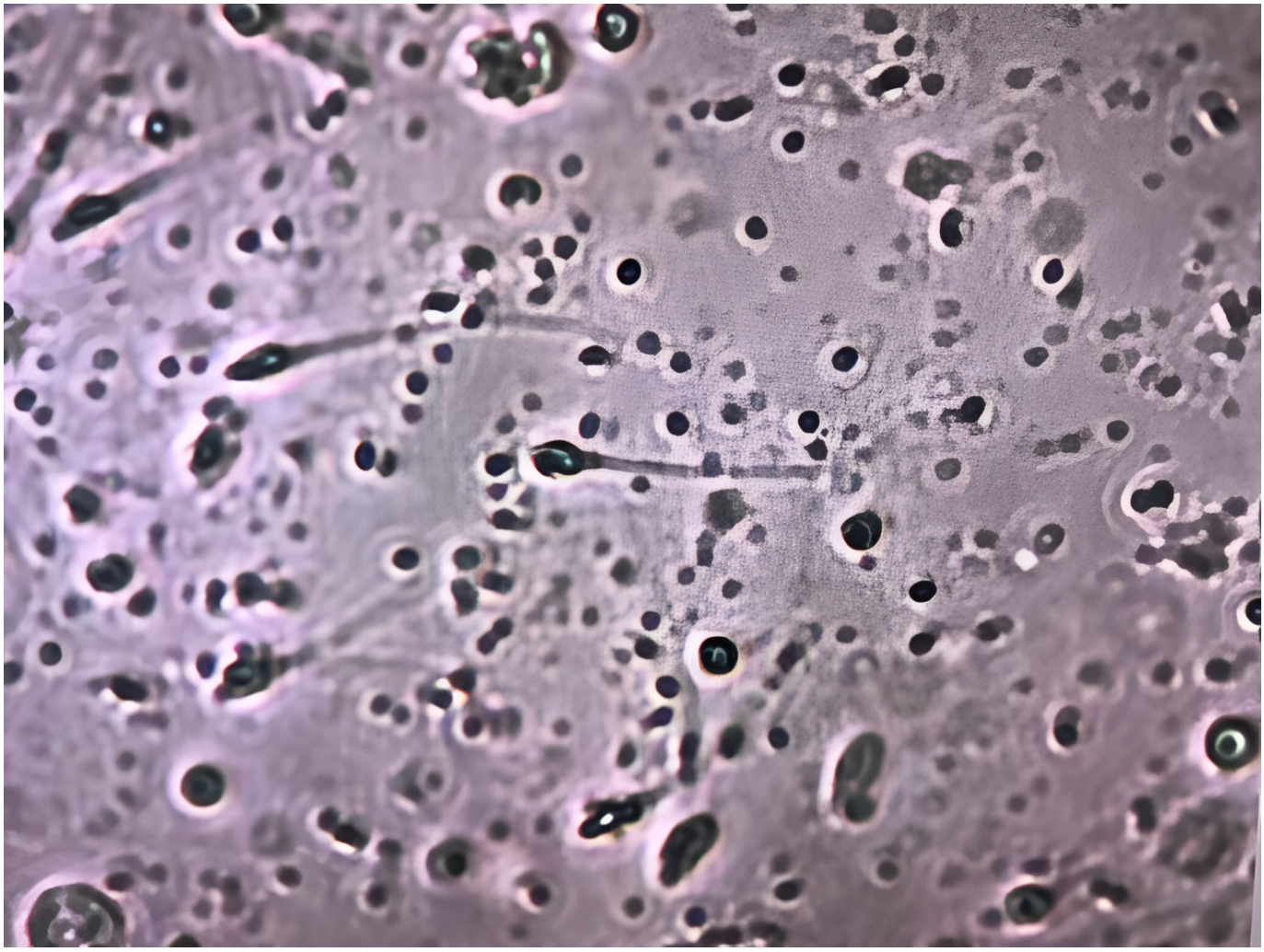

FIGURE 3. Microscopic view (20–25× magnification) of sperm in a TESE-positive patient.

2.4 Statistical analysis

The descriptive statistics of the data were expressed as means, standard deviations, maximums and minimums, medians, frequencies, and ratios. The Kolmogorov-Smirnov test and Shapiro-Wilk test were employed to examine the distribution of the variables. Independent sample *t*-tests were used for normally distributed continuous variables, while Mann-Whitney U tests were used for non-normally distributed variables to ensure appropriate statistical handling of the dataset. Additionally, a subgroup analysis was performed to compare SWE values between TESE-positive and TESE-negative patients within the KS group. To further evaluate the potential impact of KS on elastography values, we reanalyzed the data after excluding KS patients and compared the SWE values between TESE-positive and TESE-negative 46XY patients. The independent sample *t*-test and Mann-Whitney U-test were used for the analysis of quantitative independent data. The chi-squared test and, alternatively, the Fischer test when the chi-squared test conditions were not met was used in the analysis of the qualitative independent data. Additionally, we conducted a correlation analysis to examine potential relationships between SWE values and hormone levels (FSH, LH, testosterone, estradiol, and prolactin). Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 27.0 (IBM Corp., Armonk, NY, USA). The standard effect size was determined to be 0.67 with a 5% margin of error (95% Confidence Interval (CI)) and a statistical power of 80%. We decided to include at least 35 cases in each group.

3. Results

A total of 84 patients were enrolled in this study. Patients were categorized into the TESE-positive (+) and TESE-negative (−) groups after the micro-TESE procedure. Of the 84 patients, sperm retrieval was successful in 39 patients (46.43%) and unsuccessful in 45 patients (53.57%). No significant differences in age (p = 0.395), infertility duration (p = 0.964), or right (p = 0.090) and left (p = 0.137) testicle sizes were observed between the TESE (+) and TESE (−) groups. The SWE values from the upper, middle, and lower right and left did not differ significantly between the TESE (+) and TESE (−) groups (Table 1).

Klinefelter syndrome (KS) was detected in 33.3% (n = 15) of the TESE-negative patients and 12.8% (n = 5) of the TESE-positive patients. Sperm retrieval was achieved in 25% (n = 5) of the 20 KS patients included in the study (Table 2). The subgroup analysis of KS patients showed no statistically

TABLE 1. Elastography and infertility-related data of the TESE (+) and TESE (−) groups.

Variables	TESE (−) n = 45 mean ± SD or N (%)	TESE (+) n = 39 mean ± SD or N (%)	*p*-value
Age (yr)	30.5 ± 5.6	31.4 ± 5.7	0.395
Infertility duration (yr)	4.4 ± 3.7	4.1 ± 3.2	0.964
Right testicle size (cc)	32.7 ± 8.4	35.8 ± 8.6	0.090
Left testicle size (cc)	33.0 ± 8.5	35.7 ± 6.6	0.137
SWE (kPa)			
Top right	2.2 ± 1.4	1.7 ± 0.8	0.135
Right middle	2.2 ± 1.4	1.8 ± 0.7	0.454
Bottom right	2.3 ± 1.5	1.8 ± 0.8	0.201
Top left	2.0 ± 0.9	1.8 ± 0.7	0.260
Left middle	2.3 ± 1.3	1.8 ± 0.7	0.146
Bottom left	2.1 ± 1.0	1.9 ± 0.8	0.309
FSH (mIU/mL)	27.8 ± 19.0	18.6 ± 22.9	0.004
LH (mIU/mL)	13.4 ± 10.5	8.5 ± 10.0	0.001
E2 (pg/mL)	29.8 ± 11.9	36.4 ± 16.4	0.036
TT (ng/dL)	301 ± 134	367 ± 169	0.049
PRL (ng/mL)	7.3 ± 2.6	8.4 ± 3.5	0.147
Genetic			
46XY	30 (66.7%)	34 (87.2%)	0.028
47XXY	15 (33.3%)	5 (12.8%)	
Complications			
(−)	42 (93.3%)	37 (94.9%)	0.766
(+)	3 (6.7%)	2 (5.1%)	

TESE (+): TESE-positive; TESE (−): TESE-negative; SWE: shear wave elastography; FSH: follicle stimulating hormone; LH: luteinizing hormone; TT: total testosterone; E2: estradiol; PRL: prolactin; TESE: testicular sperm extraction; SD: standard deviation.

TABLE 2. SWE values for TESE-positive and TESE-negative KS patients.

SWE Measurement (kPa)	TESE-Positive KS (n = 5) Mean ± SD	TESE-Negative KS (n = 15) Mean ± SD	*p*-value
Top right	10.3 ± 0.4	10.2 ± 0.7	0.780
Middle Right	9.8 ± 0.6	9.9 ± 0.5	0.850
Bottom right	11.8 ± 0.8	11.7 ± 0.9	0.910
Top left	10.2 ± 0.7	10.3 ± 0.8	0.870
Middle Left	12.1 ± 0.9	12.0 ± 1.0	0.820
Bottom left	7.6 ± 0.8	7.7 ± 0.9	0.890

SWE: Shear wave elastography, measuring testicular stiffness in kilopascals (kPa). Comparisons between TESE-positive and TESE-negative KS patients were performed using the Mann-Whitney U test. TESE: testicular sperm extraction; KS: Klinefelter syndrome; SD: standard deviation.

significant differences in SWE values between TESE-positive and TESE-negative KS patients, with p-values ranging from $p = 0.780$ to $p = 0.910$ across different testicular regions (Table 2).

To assess the impact of KS on SWE values, the data were reanalyzed after excluding KS patients. The comparison between TESE-positive and TESE-negative 46XY patients showed that the differences in SWE values remained statistically non-significant, with p-values ranging from $p = 0.650$ to $p = 0.740$ (Table 3).

The mean SWE values were significantly higher in the KS group (10.3 ± 0.4 kPa) than in the normal genetic group (2.2 ± 1.4 kPa), with a statistically significant difference ($p < 0.001$) (Table 4). KS patients exhibited the highest stiffness, consistent with fibrotic and atrophic testicular architecture. By contrast, no statistically significant differences were observed between the KS and non-KS groups in other clinical and hormonal parameters, including FSH ($p = 0.003$), LH ($p = 0.001$), TT ($p = 0.047$), and estradiol ($p = 0.041$) (Table 4).

FSH ($p = 0.004$) and LH ($p = 0.001$) levels were significantly lower in the TESE (+) group than in the TESE (−) group. Conversely, estradiol (E2) ($p = 0.036$) and total testosterone (TT) ($p = 0.049$) levels were significantly higher in the TESE (+) group than in the TESE (−) group (Fig. 4). Correlation analysis between SWE values and hormone levels (FSH, LH, testosterone, estradiol, and prolactin) revealed no statistically significant correlations (all p-values > 0.05). No significant differences in prolactin (PRL) levels were observed between the TESE (+) and TESE (−) groups ($p = 0.147$). The postoperative complications rate did not differ significantly between the groups ($p = 0.766$).

4. Discussion

The micro-TESE surgical technique demonstrated the highest sperm retrieval success rate in patients with NOA. This method ensures the sampling of focal healthy-looking tubules, maximizing spermatozoa yield, reducing testicular tissue loss, increasing SRR, and protecting subtunical vessels. Previous studies have reported SRRs of 35–77% [13, 14]. Currently, no non-invasive clinical or laboratory parameters exist for predicting sperm retrieval before micro-TESE. Various laboratories, clinical, and imaging methods have been investigated, but no significant results have emerged from meta-analyses. Ongoing

TABLE 3. SWE values for TESE-positive and TESE-negative groups (after excluding KS patients).

SWE Measurement (kPa)	TESE-Positive KS (n = 34) Mean ± SD	TESE-Negative KS (n = 30) Mean ± SD	p-value
Top right	2.2 ± 1.4	2.3 ± 1.5	0.650
Middle Right	2.2 ± 1.4	2.2 ± 1.3	0.720
Bottom right	2.3 ± 1.5	2.1 ± 1.4	0.690
Top left	2.0 ± 0.9	2.1 ± 1.0	0.710
Middle Left	2.3 ± 1.3	2.2 ± 1.2	0.680
Bottom left	2.1 ± 1.0	2.1 ± 1.1	0.740

SWE: Shear wave elastography, measuring testicular stiffness in kilopascals (kPa). Comparisons between TESE-positive and TESE-negative non-KS (46XY) patients were performed using the Mann-Whitney U test. TESE: testicular sperm extraction; KS: Klinefelter syndrome; SD: standard deviation.

TABLE 4. Elastography and infertility-related data of the KS and non-KS groups.

Parameter	KS Group (n = 20) Mean ± SD	Non-KS Group (n = 64) Mean ± SD	p-value
Age (yr)	31.2 ± 4.8	30.9 ± 5.9	0.745
Infertility duration (yr)	4.5 ± 3.3	4.1 ± 3.6	0.652
SWE (kPa)	10.3 ± 0.4	2.2 ± 1.4	<0.001
FSH (mIU/mL)	27.6 ± 18.2	18.9 ± 16.5	0.003
LH (mIU/mL)	12.8 ± 9.8	7.6 ± 8.9	0.001
TT (ng/dL)	291.4 ± 123.5	371.2 ± 156.8	0.047
Estradiol (pg/mL)	29.9 ± 11.2	35.8 ± 14.6	0.041

SWE: shear wave elastography; FSH: follicle stimulating hormone; LH: luteinizing hormone; TT: total testosterone; SD: standard deviation.

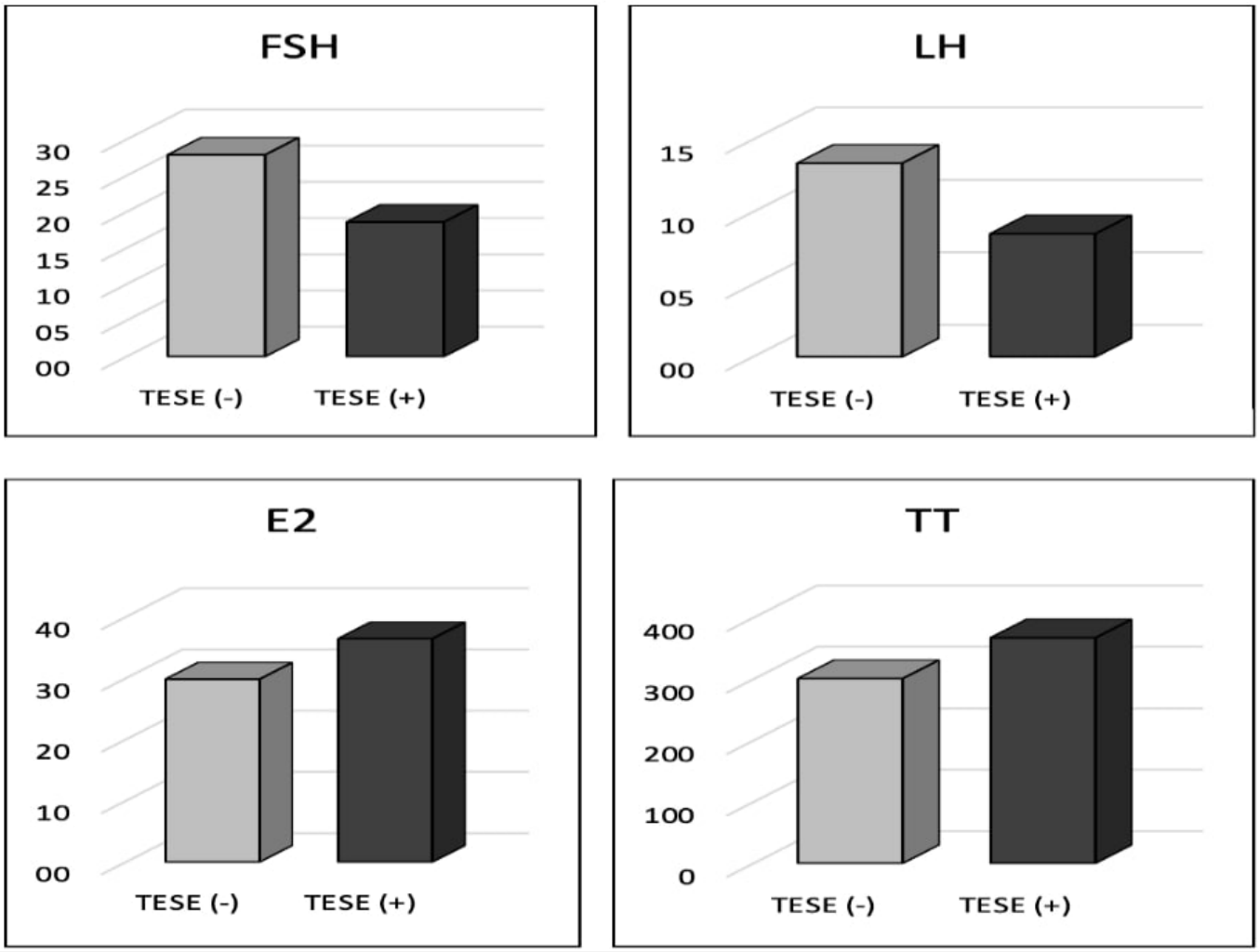

FIGURE 4. Hormone levels in the TESE (+) and TESE (−) groups. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) were higher in the TESE (−) group, and estradiol (E2) and total testosterone (TT) were higher in the TESE (+) group.

studies continue to explore parameters for predicting sperm retrieval before TESE.

Elastography comprises two techniques: SE and SWE. SE semi-quantitatively detects strain rates caused by small deformations from the ultrasound probe during the compression and decompression cycle. Recently, SWE has emerged as a promising tool for assessing viscoelastic tissue properties. Studies have demonstrated its use in evaluating scrotal masses and male infertility [15]. SWE quantitatively measures tissue stiffness (in kPa or m/s) in a target location and has been applied to various organs, including the thyroid, breasts, and liver [16, 17]. However, testicular SWE lacks standardized evaluation areas, leading to variations in reported values, with most studies focusing on testicular elasticity in cases of tumors, torsion, or infarctions [18–21]. To improve accuracy, our study obtained measurements from three separate areas in each testicle.

SWE values in infertile patients were significantly higher than those in controls, suggesting that pathological changes leading to sperm count abnormalities are associated with increased testicular stiffness. SWE has thus been proposed as a reliable technique for quantitatively measuring testicular tissue stiffness in infertility cases [8]. Illiano *et al.* [12] found a negative correlation between testicular SWE values and sperm count, linking increased stiffness to histological damage. However, their study did not exclude patients with conditions such as varicocele or testicular surgery history, which might have influenced SWE measurements.

Rocher *et al.* [22] reported significantly higher testicular stiffness in KS-NOA patients than in other infertility groups. Consistently, our study found significantly higher SWE values in KS patients compared to non-KS NOA patients. However, no significant differences in SWE values were observed between TESE-positive and TESE-negative KS patients, suggesting that increased stiffness is a general characteristic of KS rather than a predictor of sperm retrieval. To further assess the impact of KS on SWE values, we conducted an additional analysis after excluding KS patients. The comparison between TESE-positive and TESE-negative 46XY patients confirmed that SWE values remained statistically non-significant. These findings indicate that the inclusion of KS patients, who generally have higher SWE values due to testicular fibrosis, does not affect the overall relationship between SWE and sperm retrieval outcomes in non-KS NOA patients. This further

supports the conclusion that elastography alone is not a reliable predictor of sperm retrieval.

Although FNA mapping has been reported as a reliable technique for predicting spermatozoa presence in micro-TESE, it remains a minimally invasive procedure requiring intervention [23]. Similarly, no entirely non-invasive clinical or biochemical markers exist to determine sperm presence in NOA patients before micro-TESE. Previous studies have suggested that higher testicular stiffness in NOA patients may help distinguish different spermatogenic failure patterns. Abdelaal *et al*. [24] reported significantly higher SWE values in TESE-negative patients than in TESE-positive patients, particularly in cases of SCOS and spermatocyte arrest. Similarly, Li *et al*. [25] concluded that SWE could reflect underlying histopathological changes in NOA patients. However, our study did not find a significant difference in SWE values between TESE-negative and TESE-positive groups, further suggesting that elastography alone is insufficient for predicting sperm retrieval.

Another 2021 study evaluated testicular histopathology in azoospermic patients based on SWE values to indirectly predict sperm retrieval probability. The study highlighted the potential of SWE as a noninvasive method for assessing spermatogenic function by measuring testicular stiffness. Patients with average SWE levels, normal testicular volume, and hypospermatogenesis were effectively distinguished from those with SCOS and spermatogenesis arrest [26]. A 2024 study further suggested that testicular elastic modulus combined with volume could serve as a noninvasive alternative to testicular biopsy for predicting SRR before micro-TESE [27]. In contrast to these findings, our study found no significant difference in SWE values between TESE-negative and TESE-positive groups. However, as expected, SWE values in KS patients were significantly higher than those in nonobstructive azoospermic patients with a normal karyotype, likely due to the atrophic and fibrotic testicular structure observed in KS.

While SWE alone did not demonstrate significant predictive value for sperm retrieval success in our study, combining SWE with other diagnostic modalities, such as histopathological examinations or advanced imaging techniques, may improve its diagnostic reliability. Previous studies have suggested that testicular biopsy findings, including the degree of fibrosis and tubular atrophy, correlate with sperm retrieval outcomes [27]. Integrating histopathological assessment with SWE could provide a more comprehensive evaluation of testicular structure and function. Additionally, advanced imaging techniques, such as contrast-enhanced ultrasound (CEUS) or multiparametric MRI, may further enhance the predictive power of SWE by providing complementary information on testicular microvascularization and tissue characteristics [28]. However, our study did not include histopathological evaluations or additional imaging techniques, limiting our ability to assess the potential advantages of a combined diagnostic approach. Future studies should explore multimodal strategies integrating SWE, histopathology, and advanced imaging to improve sperm retrieval prediction in NOA patients.

A literature review indicated no significant relationship between preoperative hormone levels (FSH, LH or TT) and SRR. In our study, lower FSH levels and higher TT levels were associated with successful sperm retrieval. However, contrary to our data, Han Zhang *et al*. [29] found that patients with severely small testicular volumes ($\leq$5 mL) and severely high FSH levels ($\geq$24.8 mIU/mL) had the best SRR. Some studies suggest that higher serum FSH levels do not affect SRR, as testicular volume and FSH levels showed no differences between TESE-positive and TESE-negative groups in most NOA studies [30, 31]. Additionally, our study found that serum E2 levels were unexpectedly higher in TESE-positive patients. Shiraishi *et al*. [32] reported that higher serum E2 levels correlated with increased sperm retrieval rates, possibly due to increased aromatase activity.

The limitations of our study include the small sample size and the inclusion of KS patients in both the TESE-positive and TESE-negative groups, which may have influenced overall elastography values. Given the rarity of KS, conducting large-scale studies remains challenging. Since KS patients have inherently higher SWE values due to testicular fibrosis and atrophy, their inclusion in both groups could have affected the comparability of SWE values between TESE outcomes. Future studies should consider analyzing KS and non-KS NOA patients separately to minimize potential confounding effects. Another limitation is that the data collection period is not recent, which may limit the applicability of the findings to current clinical settings.

Another limitation is the lack of histopathological follow-up in TESE-positive cases. While histopathological examination was conducted for TESE-negative patients, it was not performed in TESE-positive cases. This limits our ability to correlate SWE values with specific spermatogenic patterns in patients with successful sperm retrieval. Future studies should incorporate histopathological evaluation of both TESE-positive and TESE-negative patients to strengthen the clinical relevance of SWE findings. Despite these limitations, our study provides valuable insights into the role of SWE in NOA patients. Future research should address these limitations by utilizing larger cohorts, conducting separate KS and non-KS analyses, and integrating histopathological assessments into the evaluation of TESE-positive cases.

5. Conclusions

Testicular elastography is a noninvasive and easily applicable imaging method for assessing the structure of the testis. Our findings indicate that it is not a reliable sonographic tool for predicting sperm retrieval in patients with NOA. We believe that the standardization of SWE measurements and the creation of homogeneous patients and control groups will be crucial for the advancement of this technique. To achieve more effective and definitive results, further studies involving larger populations are needed.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

AT, ESP, MD, İY—Research conception and design. AT, ESP—Data acquisition. ESP, MD, İY, BK—Data analysis and interpretation; Supervision. AT, ESP, BK—Drafting of the manuscript. AT, ESP, MD, HÇ—Critical revision of the manuscript; Statistical analysis. ESP, MD, İY, BK, HÇ—Administrative, technical or material support. AT, ESP, HÇ—Approval of the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The ethics committee approval for conducting the study was obtained from the local ethics committee (30 December 2019, Session No. 08, Decision No. 10). Informed consent was obtained from all participants prior to their inclusion in the study.

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We are saddened by the passing of Dilek Şen Dokumacı, who lost her life in a traffic accident. We express our condolences and thank her for the significant contributions she made to our work, even though we were unable to include her as an author.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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