Original Article

Retrospective cohort study of intracytoplasmic sperm injection outcomes using testicular *vs*. ejaculated sperm among patients with non-obstructive azoospermia or cryptozoospermia

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Background: Non-obstructive azoospermia (NOA) represents the most severe form of male infertility, for which microsurgical testicular sperm extraction (mTESE) is the standard treatment. Extended sperm search and micro-freeze (ESSM) offers a non-invasive alternative for identifying rare spermatozoa in ejaculate, but its clinical utility as an initial intervention prior to mTESE remains uncertain. This study compares sperm identification rates and intracytoplasmic sperm injection (ICSI) outcomes among men with NOA or cryptozoospermia who underwent ESSM as an initial intervention *vs*. those who proceeded directly to mTESE.

Methods: A retrospective cohort study of 73 male patients with NOA or cryptozoospermia severe enough to qualify for mTESE at NYU Langone Fertility Center between 2018 and 2024. Patients were categorized based on their initial intervention: ESSM-first group (n=45), in which ESSM was attempted prior to surgical retrieval, and an mTESE-first group (n=28), in which patients proceeded directly to surgery. The primary outcome was overall sperm identification rate per patient. Secondary outcomes included sperm retrieval success from mTESE after failed ESSM, and among patient proceeding to in vitro fertilization (IVF) with ICSI, fertilization rate, blastocyst formation rate, euploid rate, and live birth rate.

Results: The stepwise ESSM-first strategy was associated with a higher overall sperm identification rate compared with proceeding directly to mTESE (76% *vs*. 54%, P=0.05, borderline statistical significance), driven primarily by the cryptozoospermia subgroup, in which 100% of patients identified spermatozoa via ESSM and avoided surgery. Among patients who failed ESSM, subsequent mTESE yielded sperm retrieval rates comparable to mTESE performed as the initial intervention (42% *vs*. 54%, P=0.49). Among IVF cycles with ICSI, fertilization rates with ejaculated *vs*. testicular sperm were similar (0.42 *vs*. 0.48, P=0.15). Blastocyst formation rates were higher with ejaculated sperm (0.50 *vs*. 0.20, P=0.002), while testicular sperm exhibited a non-significant trend toward higher euploid rates (0.68 *vs*. 0.40, P=0.07). There were no significant differences in live birth rates per embryo transfer cycle ejaculated *vs*. testicular sperm cycles (54% *vs*. 46%, P=0.66).

Conclusions: In men with NOA or cryptozoospermia eligible for surgical sperm retrieval, an ESSM-first strategy was associated with higher overall sperm identification rates while preserving comparable reproductive outcomes among those proceeding to IVF with ICSI. These findings support consideration of ESSM as an initial, non-invasive strategy prior to mTESE in appropriately selected patients.

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Keywords: Non-obstructive azoospermia (NOA); cryptozoospermia; microsurgical testicular sperm extraction (mTESE); extended sperm search and micro-freeze (ESSM); intracytoplasmic sperm injection (ICSI)

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Introduction

Background

Male factor infertility accounts for 20–30% of infertility cases and contributes to up to 50% of overall infertility cases when considered as a contributing factor (1,2). Non-obstructive azoospermia (NOA), characterized by the absence of spermatozoa in ejaculate due to failed spermatogenesis, is considered the most severe form of male infertility (2,3). NOA affects approximately 1% of men and can be either congenital or acquired (4,5). Cryptozoospermia, defined as spermatozoa absent from standard fresh preparation of semen but seen in a centrifuged pellet, represents a distinct condition reflecting severely impaired spermatogenesis, with a quantitative threshold below 0.1 million/mL (2,6). NOA and cryptozoospermia share the clinical challenge of spermatozoa either absent or insufficient for conventional insemination, necessitating intracytoplasmic sperm injection (ICSI) with in vitro fertilization (IVF) (7). ICSI permits fertilization using a single spermatozoon injected directly into a mature oocyte, enabling biological paternity in cases where conventional insemination is not possible (7).

Highlight box

Key findings

- A stepwise strategy, extended sperm search and micro-freeze (ESSM)-first strategy, was associated with higher overall sperm identification rates compared with proceeding directly to microsurgical testicular sperm extraction (mTESE) (76% *vs*. 54%, P=0.05).

What is known and what is new?

- ESSM is a non-invasive alternative to mTESE for identifying rare spermatozoa in the ejaculate of patients with severe male-factor infertility.
- In this cohort, all patients with cryptozoospermia eligible for mTESE successfully identified spermatozoa using ESSM and avoided surgical retrieval. Among patients who failed ESSM, subsequent mTESE yielded sperm retrieval rates comparable to mTESE performed as the initial intervention. Spermatozoa obtained via ESSM and mTESE yielded similar fertilization rates, euploid rates, and live birth rates in *in vitro* fertilization cycles with intracytoplasmic sperm injection.

What is the implication, and what should change now?

- For men with cryptozoospermia eligible for mTESE, and ESSM-first approach is supported as the initial strategy, as it avoids surgical morbidity while preserving the option for subsequent mTESE if needed.
- For men with non-obstructive azoospermia without suspected Sertoli cell-only histology, ESSM-first may be considered a reasonable initial option to reduce surgical risk, with mTESE success rates preserved after failed ESSM.

Rationale and knowledge gap

Microsurgical testicular sperm extraction (mTESE) is the standard treatment for men with NOA and is recommended for many men with cryptoozospermia when ejaculated sperm cannot be reliably obtained for ICSI (8-10). This invasive procedure requires micro-dissection of the testes to retrieve spermatozoa (8,11). However, mTESE has a reported sperm retrieval rate of 52%, with no single, reliable preoperative predictor of retrieval success, posing a challenge to patient counseling (12-16). Despite advances in microsurgical techniques to minimize risks, mTESE still poses procedural risks including testes damage, post-operative declines in testosterone levels, and anesthesia complications (17-19). Consequently, men eligible for mTESE may opt to attempt a non-invasive sperm retrieval method first.

Extended sperm search and micro-freeze (ESSM) offers a non-invasive alternative to identify rare spermatozoa in the ejaculate of men with severely impaired spermatogenesis (20). A recent study found that nearly one in five men (19.9%) of patients initially diagnosed with NOA identified spermatozoa upon extended semen analysis (21). This technique involves dividing semen samples into micro-liter sized droplets, systematically scanning each droplet under a high-powered microscope, and cryopreserving individual spermatozoa (22). When successful, ESSM avoids the need for surgical intervention entirely.

However, questions regarding appropriate use of ESSM remain unanswered. First, direct comparison of sperm identification rates between ESSM and mTESE among men who are otherwise candidates for surgical retrieval is limited, leaving the clinical threshold for recommending ESSM-first uncertain (22). Second, when rare spermatozoa are identified in ejaculate, uncertainty persists whether ejaculated sperm from men with cryptozoospermia yield equivalent IVF outcomes compared with testicular sperm, given concerns about DNA fragmentation, impaired chromatin integrity, and reduced fertilizing potential associated with severe oligospermia (23-28). Investigating these questions has direct implications for clinical decision-making and patient counselling surrounding the benefit of attempting ESSM prior to mTESE for patients with NOA and cryptozoospermia.

Objective

The objective of this study was to evaluate a stepwise sperm retrieval strategy for men with NOA or cryptozoospermia referred for mTESE, in which ESSM is attempted prior to surgical sperm retrieval. The primary aim was to compare spermatozoa identification rates between men who attempted ESSM prior to mTESE and those who proceeded directly to mTESE. Secondary aims were to compare sperm retrieval success on rescue mTESE after failed ESSM *vs.* mTESE as the first intervention, and to compare IVF with ICSI outcomes, including fertilization rate, blastocyst formation rate, euploid rate, and live birth rate between ejaculated and testicular spermatozoa. We present this article in accordance with the STROBE reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-2026-1-0048/rc).

Methods

Study design

This retrospective cohort study analyzed data from male patients diagnosed with NOA or cryptozoospermia who underwent mTESE or ESSM at NYU Langone Fertility Center between 2018 and 2024. Data were collected from the center's electronic medical records, including semen analysis, mTESE operative report, and ESSM reports uploaded as media. The study was conducted in accordance with the Declaration of Helsinki and its subsequent amendments. The study was approved by the Institutional Review Board of New York University Grossman School of Medicine (No. #13-00389), and individual consent for this retrospective analysis was waived.

This study included all patients who were eligible for mTESE meeting inclusion criteria during the study period. The sample size was determined by the number of qualifying patients with available clinical and laboratory data. No a priori power calculation was performed.

Patients

Inclusion criteria were male patients with a diagnosis of NOA or cryptozoospermia severe enough to qualify for mTESE confirmed through two semen analyses who subsequently underwent mTESE or ESSM. Cryptozoospermia was diagnosed as the absence of spermatozoa from standard fresh preparation of semen but observed in a centrifuged pellet by an experienced andrologist, consistent with a concentration of fewer than 0.1 million/mL and insufficient for conventional insemination (29). Exclusion criteria were obstructive azoospermia, severe oligoasthenoteratozoospermia, documented female-factor infertility, or mTESE performed at an outside institution.

Participants were categorized based on the first intervention received: "mTESE first" and "ESSM first". Patients who underwent ESSM first but failed to identify spermatozoa subsequently proceeded to mTESE and were categorized as "mTESE after ESSM". Patients who pursued ESSM after undergoing mTESE were included in the "mTESE first" group, and subsequent ESSM procedures were excluded from analysis. A flow diagram depicting patient categorization is provided in *Figure 1*.

Because allocation to the first intervention was influenced by time-dependent change in institutional practice and patient preference or ability to pursue ESSM prior to mTESE, baseline demographic and clinical characteristics were compared between groups to assess comparability and potential selection bias (*Table 1*).

Before ESSM or mTESE, serum total testosterone (TT), follicle-stimulating hormone (FSH), and estradiol were measured; reference intervals used were testosterone 300–1,000 ng/dL, FSH 1.5–12.4 mIU/mL, and estradiol 10–40 pg/mL. Patients with serum TT levels <300 ng/dL underwent medical optimization for at least 3 months prior to sperm retrieval (30). Clomiphene citrate was used as first-line therapy; anastrozole was added or substituted based on the testosterone-to-estradiol ratio. "Initial" hormone values

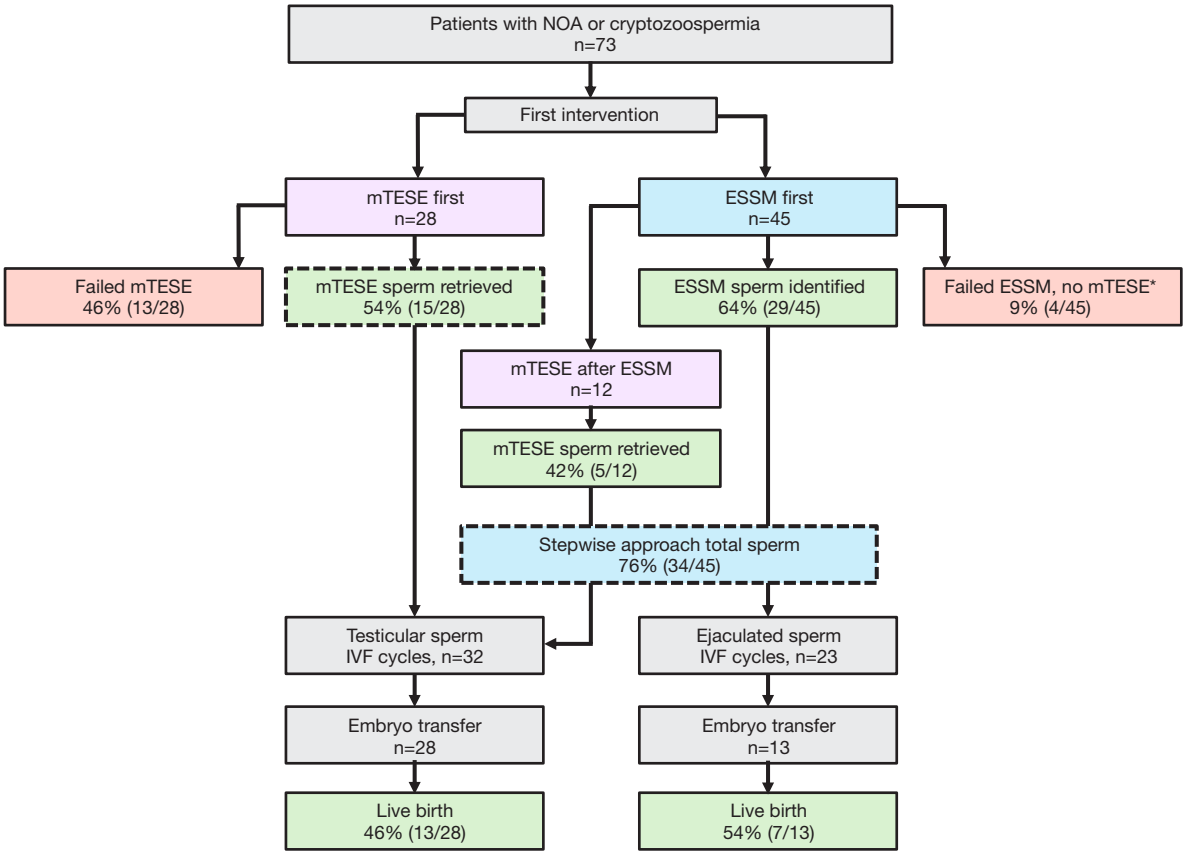

Figure 1 Protocol flow diagram for retrospective study comparing mTESE *vs.* ESSM as first intervention in men with NOA or cryptozoospermia. Dashed borders indicate the primary outcome comparison: sperm identification for the mTESE-first group *vs.* overall stepwise ESSM first approach. Patients in the ESSM first group who failed to identify spermatozoa proceeded to mTESE. *, four patients in the ESSM first group who failed ESSM did not proceed to mTESE and included as failures in ESSM first group denominator. ESSM, extended sperm search and micro-freeze; IVF, in vitro fertilization; mTESE, microsurgical testicular sperm extraction; NOA, non-obstructive azoospermia.

refer to measurements obtained at the first clinical visit prior to any medical intervention; "final" values refer to the most recent measurement following completion of optimization, or the same value as initial for patients who did not undergo medical optimization. Varicoceles were graded I–III by physical examination per World Health Organization (WHO) criteria (31). Clinical varicocele treatment was discussed and, if elected, performed via microsurgical subinguinal approach or varicocele embolization at least 3 months prior to ESSM or mTESE (7).

Interventions

Study group selection

Prior to January 1st, 2022, patients with NOA or cryptozoospermia severe enough to qualify for mTESE were routinely recommended mTESE as the first-line intervention. The "mTESE first" group includes men who proceeded with mTESE as the first-line intervention. After January 1st, 2022, ESSM was offered as a preliminary, non-invasive alternative for patients who could financially afford it. Patients electing this approach were categorized as the "ESSM first" group. If ESSM failed to identify spermatozoa, patients proceeded to mTESE and were categorized as "mTESE after ESSM".

ESSM protocol

Patients underwent ESSM at Maze Laboratories, as described by Miller *et al.* (22). Briefly, semen samples were divided into 0.5 mL aliquots and loaded onto a

Table 1 Demographics of patients undergoing mTESE first *vs*. ESSM first

Characteristics	mTESE first (n=28)	ESSM first (n=45)	P value
Age at initial visit (years)	36.3 [32.5, 39.8]; 38.8±10.2 [26.7–76.9]	35.3 [33.2, 40.5]; 37.1±6.2 [28.0–57.6]	0.96
Clinical diagnosis			0.24
NOA	72 (20/28)	58 (26/45)	
Cryptozoospermia	28 (8/28)	42 (19/45)	
Semen analysis			
Volume (mL)	3.2 [1.5, 4.3]; 3.1±1.80	2.8 [1.7, 4.3]; 3.0±0.27	0.67
pH	8.1 [8, 8.4]; 8.1±0.30 [7.4–8.6]	8.1 [8, 8.3]; 8.2±0.29 [7.4–9.0]	0.60
Total motile sperm (number)[b]	0 [0, 0]; 0.61±3.0 [0–18]	0 [0, 0]; 24.9±90.3 [0–18]	0.29[a]
Motility (%)	0 [0, 0]; [0–15]	0 [0, 0]; [0–34]	0.22[a]
Normal morphology (%)	0 [0, 0]; [0–15]	0 [0, 0]; [0–13]	0.96[a]
Genetics			0.80[a]
Y-chromosome microdeletion	7 (2/28)	4 (2/45)	
Kleinfelter syndrome	7 (2/28)	4 (2/45)	
Other[c]	0 (0/28)	3 (1/45)	
Normal	86 (24/28)	89 (40/45)	
Hormone analysis[d]			
Initial TT (ng/dL)	330 [212, 428.8]; 341.5±156.2 [61–689]	391 [275.5, 557.5]; 412.2±185.1 [112–827]	0.11
Initial FSH (mIU/mL)	19.2 [9, 26.3]; 18.3±9.9 [3.9–38.8]	17.8 [8.7, 29.1]; 20.2±13.7 [2.3–62]	0.84
Initial E2 (pg/mL)	22 [18, 30.0]; 24.0±9.8 [2.2–44]	26 [18, 31.5]; 25.5±8.8 [11.9–47]	0.59
Final TT (ng/dL)	411 [323.5, 563]; 431.4±172.4 [81–839]	469 [334, 582]; 474.8±189.8 [97–1074]	0.24
Final FSH (mIU/mL)	20.1 [10.5, 28.3]; 22.2±16.5 [4–82.6]	16.9 [8.7, 28.2]; 20.1±14.1 [2.5–68]	0.58
Final E2 (pg/mL)	24 [18.5, 40.5]; 32.4±21.6 [2.2–94]	27 [18.2, 34.8]; 27.7±10.2 [11.9–51]	0.73
Therapy[e]			
Clomiphene citrate	32 (9/28)	24 (11/45)	0.48
Anastrozole	0 (0/28)	4 (2/45)	0.26[a]
Clomiphene + anastrazole	4 (1/28)	4 (2/45)	0.86[a]
Varicocelectomy[c]	25 (7/28)	9 (4/45)	0.06
No treatment	50 (14/28)	62 (28/45)	0.30
Physical exam[f]			
Left varicocele	61 (17/28)	49 (22/45)	0.41
Right varicocele	14 (4/28)	16 (7/45)	0.84
Left testis volume (mL)	8.8 [5.4, 14]; 10.1±6.1 [2.5–27.6]	9.6 [5.8, 12.1]; 9.7±4.8 [0–19.2]	0.98
Right testis volume (mL)	8.8 [7.9, 14]; 10.4±5.0 [2–18.4]	10 [6.4, 12]; 9.7±4.4 [1.9–20.3]	0.52

Data are presented as median [IQR]; mean ± SD and [minimum–maximum] or % (n/total). [a], P values calculated by Fisher's exact test due to sparse or zero cell counts. [b], total motile sperm with normal morphology reported on semen analysis closest to initial clinical visit. [c], "Other" genetic abnormality was a Robertsonian translocation of chromosomes 13;14. [d], reference intervals: TT 300–890 ng/dL; FSH 1.0–12.0 mIU/mL; E2 11–44 pg/mL. Initial: measured prior to treatment. Final: most recent value after treatment, or same if no treatment performed. [e], numerators do not sum to denominator because patients may receive more than one treatment. [f], varicocele graded I–III by physical examination per WHO criteria. Varicocelectomies were performed via microsurgical subinguinal approach at least 3 months prior to sperm retrieval. E2, estradiol; ESSM, extended sperm search and micro-freeze; FSH, follicle-stimulating hormone; IQR, interquartile range; mTESE, microsurgical testicular sperm extraction; NOA, non-obstructive azoospermia; SD, standard deviation; TT, total testosterone; WHO, World Health Organization.

PureCeption Sperm Separation Media (Sage IVF Inc., Trumbull, CT, USA) gradient of 0.5 mL 40% upper phase and 0.5 mL 80% lower phase. Samples were centrifuged at 300 relative centrifugal force (rcf) for 20 minutes at room temperature. The supernatant was discarded, and the resulting pellet was resuspended in sperm washing modified human tubal fluid (HTF) medium with 5 mg/mL human serum albumin (Vitrolife, Gothenburg, Sweden), followed by a second centrifugation at 600 rcf of 10 minutes at room temperature. This wash step was repeated once more, after which the final pellet was resuspended in 100-µL sperm washing medium. The entire pellet was then distributed into 5-µL droplets on the lid of a 100-mm Petri dish. Each droplet was systematically examined for sperm under 100× or 200× total magnification, and any identified spermatozoa were transferred to a collection droplet. Select patients (n=5) used fresh ejaculated sperm for fertilization. For cryopreservation, a 10-µL droplet containing a 50/50 v/v mixture of Quinn's Advantage sperm freezing medium (Sage) and sperm washing medium (VitroLife) was prepared, and 0.8–1 µL droplets were placed onto each well of a SpermVD (MFC Global, Rishon LeZion, Israel).

mTESE protocol

Patients underwent mTESE in standard fashion by a single fertility-trained urologist, as originally described by Schlegel *et al*. (11). Retrieved spermatozoa were used in fresh ICSI cycles on the day of partner's oocyte retrieval, with remaining spermatozoa cryopreserved for future use. Under monitored sedation, the larger testis was delivered, and the tunica albuginea was opened approximately 270 degrees in an equatorial plane. Using an operating microscope, the seminiferous tubules were examined at 12× to 18× magnification. Microdissection of the testicular parenchyma took place superficially, and then deep into the tissue, until either spermatozoa were identified by an andrologist or a sampling of the entire testis was performed.

Ovarian stimulation protocol

Ovarian stimulation protocols were determined by the treating physician on the basis of ovarian reserve. Metaphase I (MI) oocytes were only cryopreserved if <15 metaphase II (MII) oocytes were retrieved during the same oocyte cryopreservation cycle. All protocols administered gonadotropins (recombinant FSH, human menopausal gonadotropins, or both). Follicular development was monitored by transvaginal ultrasound and serum estradiol level. A gonadotropin-releasing hormone antagonist was introduced when a lead follicle reached ≥13 mm or estradiol exceeded 1,000 pg/mL. Final oocyte maturation was triggered using either human chorionic gonadotropin alone or in combination with leuprolide acetate, as appropriate. Oocyte retrieval was scheduled approximately 35 hours after trigger administration and performed via ultrasound-guided transvaginal aspiration.

Oocyte thaw, ICSI, and preimplantation genetic testing for aneuploidy (PGT-A)

Oocyte thaw was carried out using standard laboratory protocols, and all oocytes underwent ICSI. Embryos were cultured to the appropriate stage for either transfer (day 3 or 5) or biopsy. For PGT-A cycles, trophectoderm biopsy was performed on blastocysts at day 5, 6, or 7, followed by genetic analysis using either array comparative genomic hybridization or next-generation sequencing. In fresh transfer cycles, surplus blastocysts were cryopreserved, while embryos undergoing PGT-A were refrozen after biopsy using either slow-freeze or vitrification methods. For patients returning for frozen embryo transfer, embryos were thawed according to standard laboratory procedures. The decision to pursue PGT was a shared decision between the treating physician and patient. Blastocysts are graded by embryologists according to Gardner criteria: first letter corresponds to inner cell mass quality (A, many cells, tightly packed; B, several cells, loosely grouped; C, very few cells); second letter corresponds to trophectoderm quality (a, many cells forming a cohesive layer; b, few cells forming a loose epithelium; c, very few large cells) (32).

Embryo transfer protocol

Frozen embryo transfers were conducted using either programmed (hormone replacement) or natural cycle protocols. In programmed cycles, patients received oral estradiol up-titrated from 2 to 6 mg/day for a minimum of 10 days or until the endometrium measured ≥7 mm in diameter. Progesterone in oil was then initiated, and embryo transfer was timed for a day-5 embryo, typically performed on the sixth day of progesterone exposure. In natural cycles, follicular development was monitored with transvaginal ultrasound alongside serum estradiol and progesterone levels until the dominant follicle reached 18 mm and ovulation was confirmed. Vaginal progesterone supplementation was started after ovulation, and embryo transfer was similarly scheduled on the sixth day of progesterone supplementation.

Outcomes

The primary outcome was overall spermatozoa identification rate per patient. For the "mTESE first" group, this was the proportion of patients with successful sperm retrieval via mTESE among all patients undergoing mTESE as the initial intervention. For the "ESSM first" group, the proportion of patients with successful sperm identification via ESSM among all patients undergoing ESSM as the initial intervention. The stepwise overall sperm identification rate, defined as successful sperm identification by either intervention (ESSM or subsequent rescue mTESE) among all patients in the ESSM first group, was compared with the sperm retrieval rate in the mTESE first group as the primary between-group comparison. Patients who failed ESSM and did not proceed to mTESE counted as failures in the stepwise denominator.

Secondary outcomes included: (I) sperm retrieval success among patients undergoing mTESE after failed ESSM compared with patients undergoing mTESE and the initial intervention; and (II) among patients who proceeded to IVF with ICSI, fertilization rate [two-pronuclei (2PN) embryos/ MII oocytes], blastocyst formation rate (blastocysts/ 2PN embryos), euploid rate (euploid embryos/blastocysts biopsied for PGT-A), pregnancy rate (gestational sac/ embryos transferred), and live birth rate (live births/embryos transferred), compared between ejaculated spermatozoa identified via ESSM and testicular spermatozoa retrieved via mTESE.

Prespecified subgroup analyses examined the primary outcomes by clinical diagnosis (NOA *vs.* cryptozoospermia), testosterone deficiency after hormonal optimization (<300 *vs.* ≥300 ng/dL), testicular biopsy histopathological subtypes, and use of fresh *vs.* frozen-thawed spermatozoa for ICSI.

Statistical analysis

Continuous variables were summarized as medians with interquartile ranges (IQRs) and range; where distributions were approximately normal, means with standard deviations (SDs) are also reported. Normality was assessed using Shapiro-Wilk tests. Between-group comparisons used t-tests for normally distributed variables and Mann-Whitney U tests for non-parametric data. Categorical variables were reported as frequencies and percentages and compared using Chi-squared tests or Fisher's exact tests when appropriate.

Clinically meaningful thresholds were prespecified prior to analysis, including testosterone deficiency defined as <300 ng/dL after hormonal optimization. Quantitative variables were analyzed on their original scale. To address the influence of clinical diagnosis as a confounder, a logistic regression adjusting for clinical diagnosis (NOA *vs.* cryptozoospermia) was performed for the primary outcome.

Data completeness was assessed for all variables included in primary and secondary analyses. Analyses were conducted using complete-case data without imputation. No patients were lost to follow-up, and missing outcome data reflected deliberate clinical decisions documented in the medical record. Only patients who used retrieved spermatozoa in an IVF cycle with ICSI during the study period were included in analysis of ICSI outcomes. Pre-specified subgroup analyses were performed to evaluate heterogeneity in ESSM *vs.* mTESE outcomes by clinical diagnosis, testosterone status, and histopathological subtype. Statistical significance was defined as a two-sided $P<0.05$. All analyses were performed using JMP Pro software (version 18.2.0; SAS Institute).

Results

Overview of study population

A total of 73 male patients with NOA (n=46) or cryptozoospermia severe enough to qualify for mTESE (n=27) met inclusion criteria and underwent sperm retrieval during the study period. Of these, 28 patients underwent mTESE as their first intervention (mTESE first group), and 45 patients underwent ESSM as their first intervention (ESSM first group). Among the 45 patients in the ESSM first group, 64% (29/45) successfully identified spermatozoa via ESSM and 36% (16/45) failed ESSM; of these, 12 subsequently underwent rescue mTESE. The 4 patients who did not proceed to mTESE had the following documented reasons: one elected to use donor sperm; one transferred care to an outside institution; and two discontinued care at NYU Langone Fertility Center, with female partners never receiving care at NYU Langone Fertility Center. All 4 patients are counted as failures in the ESSM-first group denominator.

Among the 49 patients who successfully retrieved sperm, 36 patients proceeded to IVF with ICSI during the study period, contributing to a total of 55 IVF cycles. The median interval between failed ESSM and subsequent rescue mTESE was 64.5 days (range, 26–125 days), and the median

interval between successful sperm retrieval and ICSI was 48 days (range, 0–1,483 days). Patient categorization by first intervention and inclusion in IVF with ICSI outcomes is summarized in *Figure 1*.

Baseline demographic and clinical characteristics of the study population are presented in *Table 1*. No significant differences were observed between the "mTESE first" and "ESSM first" groups with respect to age, diagnosis (NOA *vs.* cryptozoospermia), semen analysis parameters, genetic causes of infertility, hormone profiles, medical therapy, or testicular volume. Unless otherwise specified, complete data were available for all baseline variables. For patients who did not undergo medical optimization and had only a single hormone assessment, those values were used to represent both initial and final hormone levels.

Spermatozoa Identification: mTESE vs. ESSM

Among patients undergoing mTESE as the initial intervention, spermatozoa were successfully retrieved in 54% of patients (15/28). Among patients undergoing ESSM as the initial intervention, 64% (29/45) of patients successfully identified spermatozoa non-invasively. The median duration of sperm search during ESSM was 240 minutes (range, 120–457 minutes). Of the 16 patients in the ESSM first group who failed to identify spermatozoa, 12 subsequently underwent rescue mTESE, among whom spermatozoa were retrieved in 42% (5/12). The sperm retrieval rate for mTESE after failed ESSM was not significantly different from that of mTESE as the initial intervention (42% *vs.* 54%, P=0.49).

In the stepwise approach of attempting ESSM prior to mTESE, the overall sperm identification rate was 76% (34/45) in the ESSM-first group compared with 54% (15/28) in the mTESE-first group (P=0.05, borderline statistical significance). Among all patients in the ESSM first group, 64% (29/45) identified spermatozoa without requiring subsequent surgical intervention. Stratified by diagnosis, 38% (10/26) of patients with NOA identified spermatozoa via ESSM alone, while 100% (19/19) patients with cryptozoospermia successfully identified spermatozoa using ESSM, avoiding mTESE entirely.

To evaluate the influence of clinical diagnosis as a potential confounder, a logistic regression adjusting for diagnosis (NOA *vs.* cryptozoospermia) was performed. After adjustment, the association between ESSM-first strategy and overall sperm identification was attenuated (P=0.13). Clinical diagnosis was the dominant predictor of sperm identification success (likelihood ratio χ^2=27.40, P<0.001). These findings indicate that the overall advantage of the ESSM-first strategy is driven primarily by the cryptozoospermia subgroup, in whom ESSM universally avoids surgical retrieval. Among patients with NOA, the adjusted difference between groups did not reach statistical significance. Sperm identification outcomes by intervention group are summarized in *Table 2*.

Subgroup analyses and histopathology

Testosterone deficiency (defined as final TT <300 ng/dL after hormonal optimization) was not associated with a significant difference in sperm retrieval outcomes within either intervention group. Among patients undergoing ESSM first, sperm identification rate did not differ significantly between patients with and without testosterone deficiency [67% (8/12) final TT <300 ng/dL *vs.* 79% (26/33) final TT ≥300 ng/dL, P=0.45]. Similarly, among patients undergoing mTESE first, sperm retrieval rate did not differ significantly between patients with and without testosterone deficiency [45% (5/11) final TT <300 ng/dL *vs.* 59% (10/17) final TT ≥300 ng/dL, P=0.70]. When the stepwise ESSM-first approach was compared with mTESE alone among testosterone-deficient patients specifically, the ESSM first strategy demonstrated a higher sperm identification rate that did not reach statistical significance [67% (8/12) ESSM first *vs.* 45% (5/11) mTESE first, P=0.31]. Taken together, these findings suggest that testosterone status alone may not reliably discriminate which patients are best served by an ESSM first *vs.* mTESE first approach. FSH levels were not associated with sperm retrieval outcomes in either group.

Concurrent testicular histopathology was available for 39 patients (27 mTESE first and 12 ESSM first with subsequent mTESE). Histologic patterns included Sertoli cell-only (SCO) in 49% (19/39), hypo-spermatogenesis in 41% (16/39), and maturation arrest in 10% (4/39) (33). The distribution of histopathology across treatment groups is shown in *Table 2*. All patients with hypo-spermatogenesis successfully identified spermatozoa regardless of the intervention [100% (11/11) mTESE first *vs.* 100% (5/5) ESSM first]. Patients with SCO histology demonstrated a lower sperm identification rate via mTESE, which was not improved by the addition of ESSM [23% (3/13) mTESE first *vs.* 0% (0/6) ESSM first, P=0.20]. These findings suggest that patients with suspected SCO should be directed to mTESE rather than attempting ESSM as an initial strategy.

Table 2 Sperm identification rates for patients treated with mTESE first *vs.* ESSM first

Characteristics	Sperm identification rate		P value
	mTESE first	ESSM first	
Overall	54 (15/28)	76 (34/45)	0.05
Clinical diagnosis			
NOA	35 (7/20)	58 (15/26)	0.13
Cryptozoospermia	100 (8/8)	100 (19/19)	>0.99
Hormone analysis[b]			
Final TT <300 ng/dL	45 (5/11)	67 (8/12)	0.31
Final TT ≥300 ng/dL	59 (10/17)	79 (26/33)	0.14
Testicular biopsy histopathology			
Hypospermatogenesis[c]	100 (11/11)	100 (5/5)	>0.99[a]
Maturation arrest[d]	33 (1/3)	0 (0/1)	0.51[a]
SCO[e]	23 (3/13)	0 (0/6)	0.20[a]

Data are presented as % (n/N). P values compare sperm retrieval rate (mTESE first) *vs.* sperm identification rate (ESSM first) within each subgroup. [a], P value calculated by Fisher's exact test due to sparse cell counts. [b], final TT: most recent testosterone after medical optimization. [c], hypospermatogenesis: reduced numbers of spermatogenic cells at all developmental stages while maintaining sequence of spermatogenetic differentiation. [d], maturation arrest: cessation of spermatogenesis at a specific developmental stage. [e], SCO: complete absence of germ cells, with the seminiferous tubules lined exclusively by Sertoli cells. ESSM, extended sperm search and micro-freeze; mTESE, microsurgical testicular sperm extraction; NOA, non-obstructive azoospermia; SCO, Sertoli cell-only; TT, total testosterone.

Semen characteristics: successful mTESE vs. ESSM

Among patients with successful sperm retrieval, a single ESSM procedure yielded a median of 26 total motile spermatozoa (range, 1–540 total motile spermatozoa), whereas a single mTESE procedure yielded a median of 76 motile spermatozoa (range, 0–228,300 motile spermatozoa). The mTESE group included two outlier cases yielding 228,300 and 207,00 total motile spermatozoa, reflecting unexpectedly high testicular yields in patients with confirmed cryptozoospermia on initial semen analysis. Despite the wide range, differences in total motile sperm retrieval between ESSM and mTESE were not statistically significant (P=0.08). Post-thaw motility of spermatozoa after induction with pentoxifylline was also comparable between ESSM and mTESE. *Table 3* summarizes the semen characteristics of spermatozoa retrieved via successful mTESE and ESSM.

ICSI and embryo transfer outcomes

Of the 49 patients with successful sperm retrieval, 36 patients proceeded to IVF with ICSI during the study period, contributing to a total of 55 cycles. Of these, 58% (32/55) used testicular spermatozoa retrieved via mTESE and 42% (23/55) used ejaculated spermatozoa identified via ESSM. A single mTESE procedure supported multiple IVF cycles (median: 2 cycles; range, 1–5), whereas ESSM-identified sperm were typically used for fewer cycles (median: 1 cycle; range, 1–3). At the time of ICSI, fresh spermatozoa were used in 31% of testicular sperm cycles and 22% of ejaculated sperm cycles (P=0.20; *Table 4*). Two ejaculated sperm cycles combined fresh and frozen-thawed spermatozoa. Remaining cryopreserved sperm inventory was available at NYU Langone Fertility Center for future use in 50% (10/20) of patients using testicular sperm and 43% (7/16) of patients using ejaculated sperm, reflecting the capacity of both techniques to support multiple future IVF cycles.

Fertilization rates were similar between ejaculated and testicular spermatozoa cycles (2PN embryos/MII oocytes: 0.48 *vs.* 0.42, P=0.15). Ejaculated sperm cycles demonstrated significantly higher blastocyst formation rates (blastocysts/total 2PN embryos: 0.50 *vs.* 0.20, P=0.002). Testicular sperm cycles showed a non-significant trend toward higher euploid rates compared to ejaculated sperm (0.68 *vs.* 0.40, P=0.07). Median oocyte age was 35 years (range,

Table 3 Semen characteristics from successful mTESE and ESSM

Characteristics	Successful mTESE	Successful ESSM	P value[†]
Total motile sperm (number)[‡]	76 [5, 17,225] [1–228,300]	26 [5, 127] [0–540]	0.08
mTESE-specific characteristics			
Post-thaw mobility (%)	4.5 [0, 6.25] [0–21]	N/A	–
PD (%)[§]	7 [4, 19] [0–52]	N/A	–
ESSM-specific characteristics			
Search time (min)	N/A	240 [180, 327] [120–457]	–
Ejaculate volume (mL)	N/A	4 [2.83, 5.88] [2–6.5]	–

Data are presented as median [IQR] [minimum–maximum]. [†], P value by Mann-Whitney U test. P values not calculated (–) for variables measured in only one group. [‡], the mTESE group includes two outlier cases with 228,300 and 207,000 total motile sperm retrieved, reflecting unexpectedly high yields. All patients in both groups had confirmed cryptozoospermia or NOA on pre-retrieval semen analysis. [§], pentoxifylline (1 mM final concentration) was added to thawed samples to differentiate viable immotile spermatozoa from non-viable sperm prior to ICSI selection. ESSM, extended sperm search and micro-freeze; ICSI, intracytoplasmic sperm injection; IQR, interquartile range; mTESE, microsurgical testicular sperm extraction; N/A, not applicable; NOA, non-obstructive azoospermia; PD, post-thaw motility after pentoxifylline induction.

Table 4 IVF with ICSI outcomes for testicular vs. ejaculated spermatozoa

Outcomes	Testicular spermatozoa (n=32 cycles)	Ejaculated spermatozoa (n=23 cycles)	P value
Male age (years)	39 [34.7, 46.7], 40.0±6.7 [28.3–56.0]	36 [34.9, 43], 39.5±7.8 [29.3–58.3]	0.47
Female age (years)	35 [31.5, 38]; 34.9±4.7 [24–44]	36 [32, 40]; 35.8±5.0 [24–45]	0.49
Fresh spermatozoa	31 (10/32)	22 (5/23)	0.20
Frozen-thawed spermatozoa[†]	69 (22/32)	69 (16/23)	–
Oocytes for ICSI (number)	10 [7, 19]; 14±10 [2–37]	12 [7, 17]; 12±7 [1–25]	0.60
2PN embryos (number)	6 [3, 12]; 7±5 [1–16]	5 [1, 6]; 5±3 [0–12]	0.05
Fertilization rate	0.48 [0.38, 0.71]; 0.52±0.19 [0.17–0.88]	0.42 [0.17, 0.6]; 0.43±0.31 [0–1]	0.15
Blastocysts (number)	1 [0, 3]; 2±2 [0–9]	2 [1, 3]; 2±2 [0–6]	0.40
Blastocyst formation rate	0.2 [0, 0.5]; 0.29±0.31 [0–1]	0.5 [0.33, 0.75]; 0.57±0.28 [0–1]	0.002*
Euploid embryos (number)	1 [0, 1]; 1±2 [0–6]	0 [0, 3]; 1±2 [0–5]	0.95
Euploid rate	0.68 [0.33, 1]; 0.61±0.38 [0–1]	0.4 [0, 0.67]; 0.38±0.39 [0–1]	0.07

Data are presented as median [IQR]; mean ± SD [minimum–maximum] or % (n/total). [†], two ejaculated sperm cycles combined fresh and frozen-thawed spermatozoa. *, P<0.05. Fertilization rate: 2PN embryos/total MII oocytes fertilized; blastocyst formation rate: blastocysts/total 2PN embryos; euploid rate: euploid embryos/blastocysts biopsied for PGT-A. Cycles without PGT-A excluded from euploid rate denominator. 2PN, two-pronuclei; ICSI, intracytoplasmic sperm injection; IQR, interquartile range; IVF, in vitro fertilization; MII, metaphase II; PGT-A, preimplantation genetic testing for aneuploidy; SD, standard deviation.

24–45 years) and did not differ significantly between groups (35 *vs.* 36 years, P=0.49). No female partners had a documented female-factor infertility diagnosis. IVF with ICSI outcomes are detailed in *Table 4*.

Fresh spermatozoa cycles demonstrated higher fertilization rates compared with frozen-thawed cycles (0.75 *vs.* 0.43, P=0.01), with no significant differences in blastocyst formation (0.33 *vs.* 0.33, P=0.71) or euploid rates (0.58 *vs.* 0, P=0.18).

A total of 42 embryo transfer cycles were performed. One testicular sperm cycle in which two aneuploid embryos were transferred was excluded from outcome analyses, as this transfer reflected patient preference in the setting

of poor prognosis, leaving 41 cycles for analysis: 28 used testicular spermatozoa and 13 used ejaculated spermatozoa. Embryos were graded by Gardner criteria at the time of cryopreservation. Single euploid blastocysts were transferred in most cycles [71% (20/28) testicular *vs.* 77% (10/13) ejaculated]. Exceptions were as follows, reflecting shared decision-making between the treating physician and patient: five testicular sperm cycles transferred two untested fresh embryos; three testicular sperm cycles transferred a single untested, frozen embryo; one ejaculated sperm cycle transferred a single untested, fresh embryo; one ejaculated sperm cycle transferred three untested, fresh embryos; one ejaculated sperm cycle transferred one low-level mosaic embryo. Blastocyst grade at cryopreservation and PGT-A testing details by sperm source are summarized in *Table 5*.

There was no significant difference in live birth rates per FET cycle between testicular and ejaculated sperm cycles [46% (13/28) *vs.* 54% (7/13), P=0.66]. Accounting for cycles in which more than one embryo was transferred, live birth rates per embryo transferred were also comparable between groups [39% (13/33) *vs.* 47% (7/15), P=0.83]. Transfer outcomes by sperm source are detailed in *Table 5*.

Discussion

Key findings

In this retrospective cohort of men with NOA or cryptozoospermia eligible for mTESE, a stepwise strategy of attempting ESSM prior to mTESE was associated with a higher overall sperm identification rate compared with proceeding directly to mTESE alone (76% *vs.* 54%, P=0.05, borderline statistical significance). While this difference did not meet the predefined threshold for statistical significance (P<0.05), the 22% absolute difference represents a clinically meaningful improvement. In a logistic regression adjusting for clinical diagnosis, the group effect attenuated and did not reach statistical significance after adjustment (P=0.13). Clinical diagnosis was the dominant predictor of sperm identification success (likelihood ratio χ^2=27.40, P<0.001), supporting that the overall advantage of the ESSM-first strategy is driven primarily by the cryptozoospermia subgroup, among whom 100% of patients identified spermatozoa via ESSM and avoided surgery entirely. Among patients who failed ESSM, subsequent mTESE yielded sperm retrieval rates comparable to mTESE performed as the initial intervention (42% *vs.* 54%, P=0.49), supporting that trial of ESSM does not compromise subsequent surgical retrieval success.

With respect to reproductive outcomes, fertilization rates and live birth rates were not different between IVF cycles using ejaculated and testicular spermatozoa. While testicular sperm demonstrated a trend toward higher euploid rates and ejaculated sperm exhibited higher blastocyst formation rates, these differences did not translate into clinically meaningful differences in embryo transfer outcomes.

Collectively, these findings support ESSM as an initial strategy for men with cryptozoospermia and a reasonable first-line option for selected men with NOA, while the surgical option is preserved for those who require it. The stepwise strategy therefore facilitates shared decision-making by offering patients a meaningful opportunity to avoid surgery, while preserving the surgical option, and may reduce overall procedural burden and cost for those who succeed non-invasively.

Strengths and limitations

Strengths of this study include the relatively large cohort of men with NOA or cryptozoospermia severe enough to qualify for mTESE, the use of standardized laboratory protocols, and performance of all mTESE procedures by a single fertility-trained urologist, reducing procedural and inter-operator variability known to influence sperm retrieval outcomes (34). The conduct of all ICSI cycles at a single fertility center enabled detailed ascertainment of embryo transfer and reproductive outcome data from sperm retrieval through live birth. All ESSM procedures were performed at a single dedicated external laboratory (Maze Laboratories, Maze Sexual and Reproductive Health, New York, NY, USA) with specific expertise in extended sperm search techniques, ensuring consistency in ESSM methodology across the cohort.

Several limitations warrant consideration. First, the retrospective, non-randomized design introduces the potential for selection bias and residual confounding. Group allocation was driven primarily by a time-dependent change in institutional practice rather than random allocation: before January 1st, 2022, mTESE was recommended as the first-line intervention; after that date, ESSM was offered as an alternative. Although baseline demographic and clinical characteristics were well balanced between groups (*Table 1*), unmeasured confounding related to calendar year, patient preference, or financial considerations may remain. Additionally, patients with cryptozoospermia who had prior knowledge of rare spermatozoa in their ejaculate after

Table 5 FET outcomes of cycles using testicular vs. ejaculated spermatozoa

Outcomes	Testicular spermatozoa	Ejaculated spermatozoa	P value[†]
Embryo characteristics at cryopreservation			
PGT-A tested			0.35
Euploid	100 (20/20)	91 (10/11)	
Low-level mosaic[‡]	0 (0/20)	9 (1/11)	
Untested			>0.99
Fresh	63 (5/8)	100 (2/2)	
Frozen	38 (3/8)	0 (0/2)	
Blastocyst day			
Day 3	0 (0/28)	15 (2/13)	0.10
Day 5	71 (20/28)	38 (5/13)	0.08
Day 6	25 (7/28)	31 (4/13)	0.72
Day 7	4 (1/28)	15 (2/13)	0.23
Blastocyst grade[§]			
Ab or Ba	4 (1/26)	9 (1/11)	0.51
Bb	69 (18/26)	82 (9/11)	0.69
Bc or Cb	27 (7/26)	9 (1/11)	0.39
Number embryos transferred			0.64
Single	82 (23/28)	92 (12/13)	
Double or triple[¶]	18 (5/28)	8 (1/13)	
Overall FET outcomes			
Live birth, per FET cycle	46 (13/28)	54 (7/13)	0.66
Live birth, per embryo transferred	39 (13/33)	47 (7/15)	0.59
SAB <20 weeks	4 (1/28)	0 (0/13)	0.49
Biochemical	14 (4/28)	8 (1/13)	0.55
Negative	35 (10/28)	38 (5/13)	0.86

Data are presented as % (n/total). Blastocyst grade assessed by Gardner criteria at time of cryopreservation (A, B, or C assigned to inner cell mass and trophectoderm morphology). [†], P value calculated by Fisher's exact test due to sparse cell counts. [‡], low-level mosaic embryos were included as per institutional practice due to similar live birth potential of low-level mosaic and euploid embryos. Transfer of two aneuploid embryos excluded from all outcome analyses as this transfer reflected patient preference in the setting of poor prognosis. [§], Gardner grading criteria; first letter corresponds to inner cell mass quality (A, many cells, tightly packed; B, several cells, loosely grouped; C, very few cells); second letter corresponds to trophectoderm quality (a, many cells forming a cohesive layer; b, few cells forming a loose epithelium; c, very few large cells) (32); embryo grading not available for two testicular and two ejaculated fresh embryo transfers. [¶], one ejaculated sperm transfer of 3 fresh, untested embryos. FET, frozen embryo transfer; PGT-A, preimplantation genetic testing for aneuploidy; SAB, spontaneous abortion.

centrifugation may have been inclined to elect ESSM first, representing a potential source of selection bias that cannot be fully controlled in a retrospective design. Second, while a logistic regression adjusting for clinical diagnosis was performed, the sample size (n=73, with subgroups as small as 8–12 patients) precluded simultaneous adjustment for all relevant confounders. The prespecified subgroup analyses by diagnosis, testosterone status, and histopathology

represent the analytic approach feasible within this sample size and should be interpreted cautiously. Third, IVF with ICSI outcome data were available for only 36 of the 49 patients with successful sperm retrieval, as over one-quarter had patients had not yet utilized sperm at the time of analysis and nearly half of those who did complete ICSI still have cryopreserved inventory remaining, limiting definitive conclusions on pregnancy outcomes

Explanation of findings in the context of similar research

Few studies have directly compared sperm identification rates between ESSM and mTESE in patients otherwise referred for surgical retrieval (22). Our overall sperm identification rate of 64% with ESSM and 54% with mTESE is somewhat lower than the 78% and 68% reported by Miller *et al.* (22), potentially reflecting differences in patient selection or inclusion of men with more severe spermatogenesis impairment. Nevertheless, the finding that 100% of patients with cryptozoospermia identified spermatozoa via ESSM is consistent with Miller *et al.*'s reported results and reinforces ESSM as the preferred initial strategy in this subgroup, while supporting the continued role of mTESE for men with NOA. Our study extends prior work by characterizing the relationship between testicular histopathology and ESSM outcome and finds that hypo-spermatogenesis was universally associated with ESSM success, while ESSM uniformly fails in patients with SCO syndrome.

Avoiding mTESE carries additional significance for men with NOA, who are more likely to experience testosterone deficiency at baseline (8) and face a risk of post-operative testosterone deficiency requiring long-term testosterone replacement therapy (8,12,17,18). In our cohort, testosterone deficiency did not significantly discriminate sperm identification success within either the ESSM first (67% final TT <300 ng/dL *vs.* 79% final TT ≥300 ng/dL, P=0.45) or mTESE first group (45% final TT <300 ng/dL *vs.* 59% final TT ≥300 ng/dL, P=0.70), and the stepwise approach demonstrated a numerically higher but non-significant identification rate among testosterone-deficient patients (67% *vs.* 45%, P=0.31). These findings are in contrast to prior studies suggesting lower mTESE retrieval rates in hypogonadal patients (35,36), and suggest that hormone status alone may not reliably guide the choice between ESSM first and mTESE first in clinical practice.

An additional novel finding was the correlation between histopathological diagnosis and ESSM success. Consistent with established literature on TESE outcomes (7,37,38), histopathological patterns influenced sperm identification success via ESSM. All patients with hypo-spermatogenesis identified spermatozoa via ESSM. In contrast, no patients with SCO syndrome identified spermatozoa with ESSM, and the mTESE retrieval rate in this subgroup was 23% (3/13), consistent with published rates of 20–40% for SCO in the literature (39-41). Although diagnostic testicular biopsy is not routinely recommended in the workup of these patients, these histopathological associations suggest that when biopsy data are available (for example, from a prior clinical evaluation), they may inform patient selection for ESSM *vs.* direct mTESE. Specifically, patients with suspected SCO should consider bypassing ESSM in favor of mTESE, as it remains their best option for sperm retrieval.

With respect to reproductive outcomes, this study contributes to the ongoing clinical debate over the efficacy of testicular *vs.* ejaculated spermatozoa for fertilization in IVF with ICSI. Spermatozoa from men with severe oligospermia may exhibit elevated DNA fragmentation (42-44), reduced chromatin integrity, and increased susceptibility to reactive oxygen species (45). Elevated DNA fragmentation index is associated with reduced fertilization rates in ART (44). Testicular spermatozoa circumvent insults that occur during transit through the reproductive tract (26,28,46). However, testicular spermatozoa also bypass epididymis maturation, which conversely may impair fertilizing ability (47,48). Prior meta-analyses provide conflicting recommendations: Kang *et al.* reported improved pregnancy rates with testicular spermatozoa in men with cryptozoospermia (23), while Abhyankar *et al.* found no significant advantages (49). A 2023 meta-analysis found that testicular sperm exhibited higher pregnancy and live birth rates than ejaculated sperm in patients with high DNA fragmentation (50). However, among patients without a history of failed IVF or evidence of DNA fragmentation, Kendall Rauchfuss *et al.* found that using testicular spermatozoa instead of ejaculated spermatozoa may be detrimental to pregnancy outcomes (24).

In our cohort, fertilization rates and live birth rates were comparable between testicular and ejaculated spermatozoa cycles (*Tables 4,5*), consistent with Miller *et al.*'s findings of similar fertilization, pregnancy, and live birth rates between sperm sources (22). Miller *et al.* report a lower spontaneous abortion rate among transfers without PGT-A with testicular spermatozoa [8.6% (3/35) *vs.* 52% (14/27), P=0.002] that was not observed in our cohort of mostly euploid embryo transfers [4% (1/28) *vs.* 0% (0/13),

P=0.49]. The significantly higher blastocyst formation rate with ejaculated spermatozoa (0.50 *vs.* 0.20, P=0.002) likely reflects patient selection rather than intrinsic sperm source effect: the ejaculated sperm group was enriched with cryptozoospermic patients who succeeded on initial ESSM, whereas the testicular sperm group was predominated by men with more severely impaired spermatogenesis who required mTESE (51). This difference is unlikely to be clinically meaningful, as euploid rates and embryo transfer outcomes remained comparable between groups.

Generalizability and clinical implications

These results are most generalizable to men with NOA or cryptozoospermia evaluated at fertility centers with access to a specialized ESSM laboratory. In this cohort, ESSM was performed at Maze Laboratories (Maze Sexual and Reproductive Health), a dedicated external facility with specific expertise in extended sperm search techniques. Given the search duration up to 7.6 hours, the ESSM-first strategy as practiced here required referral to a specialized partner laboratory. Outcomes may not be reproducible in settings without equivalent access, and centers seeking to implement an ESSM-first approach should consider developing such partnerships or establishing equivalent in-house capabilities. Outcomes may also differ based on surgical experience for mTESE and the underlying characteristics of the patient population.

From a clinical perspective, a stepwise ESSM-first strategy offers patients a non-invasive opportunity to identify spermatozoa before undergoing surgery, reducing procedural risk and potentially lowering cost for those who succeed, while preserving the option of mTESE for those who do not. The financial burden of ESSM and mTESE warrants consideration. A single ESSM costs $2,000, while mTESE procedure may exceed $10,000 depending on the center. In our cohort, 22% of patients treated with ESSM required more than one extended search, with one patient undergoing six ESSM to complete family building. For patients who fail multiple ESSM attempts before proceeding to mTESE, cumulative costs may exceed those of initial mTESE. These expenses must be understood in the context of Mehta *et al.*'s study reporting that 46% of men seeking fertility care had treatment options limited by cost (52). Future cost-effectiveness studies are needed to optimize patient selection for ESSM *vs.* mTESE and help guide shared decision-making from a financial planning perspective.

Implications and actions needed

Randomized prospective studies comparing ESSM-first *vs.* mTESE-first strategies with random allocation and standardized follow-up through embryo transfer and live birth are needed to confirm these findings. Formal cost-effectiveness studies incorporating procedure costs and IVF success rates are needed to help inform patient selection for ESSM-first *vs.* mTESE-first and guide shared-decision making from a financial planning perspective. Together, these studies would provide the evidence base necessary to develop algorithmic decision support for clinicians managing men with NOA or cryptozoospermia.

Conclusions

In men with NOA or cryptozoospermia eligible for surgical sperm retrieval, a stepwise ESSM-first strategy was associated with higher overall sperm identification rates compared with mTESE alone (76% *vs.* 54%, P=0.05), a difference of borderline statistical significance representing a clinically meaningful 22% absolute improvement, driven primarily by the cryptozoospermic subgroup in whom ESSM uniformly succeeded in identifying spermatozoa non-invasively. Among patients who failed ESSM, subsequent mTESE preserved comparable sperm retrieval success, and ejaculated and testicular spermatozoa yielded equivalent reproductive outcomes. These findings support consideration of ESSM as an initial strategy in appropriately selected patients and highlight the need for prospective evaluation of stepwise sperm retrieval pathways.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-2026-1-0048/rc

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki and its subsequent amendments. The study was approved by the Institutional Review Board of New York University Grossman School of Medicine (No. #13-00389), and individual consent for this retrospective analysis was waived.

References

1. Magoutas K, Leathersich S, Hart R, et al. Lower Semen Quality Among Men in the Modern Era-Is There a Role for Diet and the Microbiome? Microorganisms 2025;13:147.
2. Agarwal A, Baskaran S, Parekh N, et al. Male infertility. Lancet 2021;397:319-33.
3. Kang C, Punjani N, Schlegel PN. Reproductive Chances of Men with Azoospermia Due to Spermatogenic Dysfunction. J Clin Med 2021;10:1400.
4. Krausz C, Cioppi F. Genetic Factors of Non-Obstructive Azoospermia: Consequences on Patients' and Offspring Health. J Clin Med 2021;10:4009.
5. Wang X, Nie L, Hong Z, et al. Genetic and epigenetic insights into non-obstructive azoospermia: mechanisms, biomarkers, and clinical perspectives. Reprod Biol Endocrinol 2025;23:159.
6. Schülke LC, Wistuba J, Nordhoff V, et al. Identification of two hidden clinical subgroups among men with idiopathic cryptozoospermia. Hum Reprod 2024;39:892-901.
7. Practice Committee of the American Society for Reproductive Medicine. Electronic address: asrm@asrm. Management of nonobstructive azoospermia: a committee opinion. Fertil Steril 2018;110:1239-45.
8. Brannigan RE, Hermanson L, Kaczmarek J, et al. Updates to Male Infertility: AUA/ASRM Guideline (2024). J Urol 2024;212:789-99.
9. Alkandari MH, Moryousef J, Phillips S, et al. Testicular Sperm Aspiration (TESA) or Microdissection Testicular Sperm Extraction (Micro-tese): Which Approach is better in Men with Cryptozoospermia and Severe Oligozoospermia? Urology 2021;154:164-9.
10. Alkandari MH, Bouhadana D, Zini A. Is a contralateral testicular exploration required at microdissection testicular sperm extraction for men with nonobstructive azoospermia, cryptozoospermia or severe oligozoospermia? Andrologia 2021;53:e14208.
11. Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. Hum Reprod 1999;14:131-5.
12. Bernie AM, Mata DA, Ramasamy R, et al. 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;104:1099-103.e1-3.
13. Aljubran A, Safar O, Elatreisy A, et al. Factors predicting successful sperm retrieval in men with nonobstructive Azoospermia: A single center perspective. Health Sci Rep 2022;5:e727.
14. Bernie AM, Ramasamy R, Schlegel PN. Predictive factors of successful microdissection testicular sperm extraction. Basic Clin Androl 2013;23:5.
15. Gao S, Yang X, Xiao X, et al. Outcomes and affecting factors for ICSI and microTESE treatments in nonobstructive azoospermia patients with different etiologies: A retrospective analysis. Front Endocrinol (Lausanne) 2022;13:1006208.
16. Ceyhan E, Kayra MV, Gul Ates E, et al. A Nomogram Predicting Testicular Sperm Extraction Success in Men With Non-obstructive Azoospermia: A Multi-center Study. Urology 2025;196:155-61.

17. Ramasamy R, Yagan N, Schlegel PN. Structural and functional changes to the testis after conventional versus microdissection testicular sperm extraction. Urology 2005;65:1190-4.
18. Eliveld J, van Wely M, Meißner A, et al. The risk of TESE-induced hypogonadism: a systematic review and meta-analysis. Hum Reprod Update 2018;24:442-54.
19. Herndon CC, Godart ES, Turek PJ. Testosterone levels among non-obstructive azoospermic patients 2 years after failed bilateral microdissection testicular sperm extraction: a nested case-cohort study. J Assist Reprod Genet 2022;39:1297-303.
20. Ron-El R, Strassburger D, Friedler S, et al. Extended sperm preparation: an alternative to testicular sperm extraction in non-obstructive azoospermia. Hum Reprod 1997;12:1222-6.
21. Sahin KC, Sutcuoglu S, Zara HR, et al. Cryptozoospermia in the Shadow of Azoospermia: Accurate Diagnosis With Clinical Predictors and Extended Semen Analysis. Urology 2025;205:82-8.
22. Miller N, Biron-Shental T, Pasternak Y, et al. Fertility outcomes after extended searches for ejaculated spermatozoa in men with virtual azoospermia. Fertil Steril 2017;107:1305-11.
23. Kang YN, Hsiao YW, Chen CY, et al. Testicular sperm is superior to ejaculated sperm for ICSI in cryptozoospermia: An update systematic review and meta-analysis. Sci Rep 2018;8:7874.
24. Kendall Rauchfuss LM, Kim T, Bleess JL, et al. Testicular sperm extraction vs. ejaculated sperm use for nonazoospermic male factor infertility. Fertil Steril 2021;116:963-70.
25. Awaga HA, Bosdou JK, Goulis DG, et al. Testicular versus ejaculated spermatozoa for ICSI in patients without azoospermia: A systematic review. Reprod Biomed Online 2018;37:573-80.
26. Najari BB, Thirumavalavan N. Should we use testicular sperm for intracytoplasmic sperm injection in all men with significant oligospermia? Fertil Steril 2021;116:971-2.
27. Esteves SC, Sánchez-Martín F, Sánchez-Martín P, et al. Comparison of reproductive outcome in oligozoospermic men with high sperm DNA fragmentation undergoing intracytoplasmic sperm injection with ejaculated and testicular sperm. Fertil Steril 2015;104:1398-405.
28. Xie P, Keating D, Parrella A, et al. Sperm Genomic Integrity by TUNEL Varies throughout the Male Genital Tract. J Urol 2020;203:802-8.
29. Tournaye H, Camus M, Goossens A, et al. Recent concepts in the management of infertility because of non-obstructive azoospermia. Hum Reprod 1995;10 Suppl 1:115-9.
30. Reifsnyder JE, Ramasamy R, Husseini J, et al. Role of optimizing testosterone before microdissection testicular sperm extraction in men with nonobstructive azoospermia. J Urol 2012;188:532-6.
31. Practice Committee of the American Society for Reproductive Medicine; Society for Male Reproduction and Urology. Report on varicocele and infertility: a committee opinion. Fertil Steril 2014;102:1556-60.
32. Gardner DK, Lane M, Stevens J, et al. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil Steril 2000;73:1155-8.
33. Hentrich A, Wolter M, Szardening-Kirchner C, et al. Reduced numbers of Sertoli, germ, and spermatogonial stem cells in impaired spermatogenesis. Mod Pathol 2017;30:311.
34. Ishikawa T, Nose R, Yamaguchi K, et al. Learning curves of microdissection testicular sperm extraction for nonobstructive azoospermia. Fertil Steril 2010;94:1008-11.
35. Esteves SC, Achermann APP, Miyaoka R, et al. Clinical factors impacting microdissection testicular sperm extraction success in hypogonadal men with nonobstructive azoospermia. Fertil Steril 2024;122:636-47.
36. Achermann APP, Esteves SC. Prevalence and clinical implications of biochemical hypogonadism in patients with nonobstructive azoospermia undergoing infertility evaluation. F S Rep 2024;5:14-22.
37. Sousa M, Cremades N, Silva J, et al. Predictive value of testicular histology in secretory azoospermic subgroups and clinical outcome after microinjection of fresh and frozen-thawed sperm and spermatids. Hum Reprod 2002;17:1800-10.
38. Su LM, Palermo GD, Goldstein M, et al. Testicular sperm extraction with intracytoplasmic sperm injection for nonobstructive azoospermia: testicular histology can predict success of sperm retrieval. J Urol 1999;161:112-6.
39. Hussein A. Evaluation of diagnostic testis biopsy and the repetition of testicular sperm extraction surgeries in infertility patients. Fertil Steril 2013;100:88-93.
40. Caroppo E, Colpi GM. Prediction Models for Successful Sperm Retrieval in Patients with Non-Obstructive Azoospermia Undergoing Microdissection Testicular Sperm Extraction: Is There Any Room for Further Studies? J Clin Med 2021;10:5538.
41. Flannigan R, Bach PV, Schlegel PN. Microdissection testicular sperm extraction. Transl Androl Urol 2017;6:745-52.

42. Esteves SC. Novel concepts in male factor infertility: clinical and laboratory perspectives. J Assist Reprod Genet 2016;33:1319-35.
43. Spanò M, Bonde JP, Hjøllund HI, et al. Sperm chromatin damage impairs human fertility. The Danish First Pregnancy Planner Study Team. Fertil Steril 2000;73:43-50.
44. Bibi R, Jahan S, Razak S, et al. Protamines and DNA integrity as a biomarkers of sperm quality and assisted conception outcome. Andrologia 2022;54:e14418.
45. Guz J, Gackowski D, Foksinski M, et al. Comparison of oxidative stress/DNA damage in semen and blood of fertile and infertile men. PLoS One 2013;8:e68490.
46. Esteves SC, Roque M, Bradley CK, et al. Reproductive outcomes of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with high levels of DNA fragmentation in semen: systematic review and meta-analysis. Fertil Steril 2017;108:456-467.e1.
47. Sullivan R, Mieusset R. The human epididymis: its function in sperm maturation. Hum Reprod Update 2016;22:574-87.
48. Barrachina F, Battistone MA, Castillo J, et al. Sperm acquire epididymis-derived proteins through epididymosomes. Hum Reprod 2022;37:651-68.
49. Abhyankar N, Kathrins M, Niederberger C. Use of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with cryptozoospermia: a meta-analysis. Fertil Steril 2016;105:1469-1475.e1.
50. Zhao G, Jiang X, Zheng Y, et al. Outcomes comparison of testicular versus ejaculated sperm for intracytoplasmic sperm injection in infertile men with high DNA fragmentation: updated systematic review and meta-analysis. Transl Androl Urol 2023;12:1785-802.
51. Chiba K, Enatsu N, Fujisawa M. Management of non-obstructive azoospermia. Reprod Med Biol 2016;15:165-73.
52. Mehta A, Nangia AK, Dupree JM, et al. Limitations and barriers in access to care for male factor infertility. Fertil Steril 2016;105:1128-37.

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