Micro-dissection testicular sperm extraction as an alternative for sperm acquisition in the most difficult cases of Azoospermia: Technique and preliminary results in India

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ABSTRACT

CONTEXT: Non-obstructive azoospermia (NOA) is an unfavorable prognostic condition for male infertility since spermatogenesis is disrupted. Sperm retrieval (SR) coupled with intracytoplasmic sperm injection (ICSI) is the only option for men with NOA who seek fertility. Among the SR techniques, microdissection testicular sperm extraction (micro-TESE) has been applied with encouraging results. **AIMS:** We describe how we implemented the micro-TESE procedure and present initial micro-TESE experience in a group of men with NOA and poor prognosis for SR. **SETTINGS AND DESIGN:** Case series of men with NOA treated in a tertiary healthcare center. **MATERIALS AND METHODS:** An Assisted Reproductive Technology (ART) facility was setup to perform SR using microsurgery. Fourteen men with NOA and previous failed retrievals or unfavorable histologic results underwent micro-TESE while their female partners received ovarian stimulation for oocyte pickup (OCP). Micro-TESE was performed the day prior to OCP and testicular sperm were used for sperm injections. We assessed retrieval rates and ICSI outcomes. **STATISTICAL ANALYSIS:** Outcomes of SR and ICSI were analyzed descriptively. Mann-Whitney and Fisher exact test were used to compare characteristics of men with successful and failed SR. **RESULTS:** The success of micro-TESE was 50.0% with no major complications. A clear microscopic distinction between enlarged and collapsed seminiferous tubules was seen in 35.7% of the cases, and sperm were retrieved in all but one of these cases. Patients with successful and failed retrieval did not differ with respect to baseline characteristics, use of medical therapy, presence of varicocele, and testicular histology. Sperm injections resulted in normal fertilization and embryo cleavage of 64% and 75%, respectively. A total of five transfers with an average of 1.5 embryos resulted in a cumulative clinical pregnancy rate per ICSI cycle of 28.6%, with an implantation rate of 33.3%. **CONCLUSIONS:** We were successful in integrating the micro-TESE procedures to the *in vitro* fertilization (IVF) laboratory. Our initial experience with micro-TESE applied to the most difficult cases of azoospermia is reassuring.

KEY WORDS: Assisted reproductive techniques, azoospermia, male infertility, microsurgery, sperm retrieval techniques, spermatozoa, sterility, testis

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INTRODUCTION

Microdissection Testicular Sperm Extraction (micro-TESE) is a surgical method of sperm acquisition for men with nonobstructive azoospermia (NOA) seeking fertility. Men with NOA have highly dysfunctional testes, and although the overall picture is of testicular failure, rare foci of sperm production may exist in up to 60% of these individuals.[1,2] Sperm production, if existent, is minimal for sperm appearance in the ejaculate, and since there are no treatment options to restore fertility in these men, the only alternative is to attempt sperm retrieval (SR) with the aim of finding viable testicular sperm to be used for *in vitro* fertilization (IVF).[3]

The method of choice to retrieve sperm in NOA has been testicular sperm extraction (TESE), with variable success rates of 25-

60% of cases.[3-7] In TESE, open single or multiple testicular biopsies are randomly obtained, and the excised testicular parenchyma is processed and examined for the presence of sperm. Due to the fact that both the existence and the geographic location of islets of normal spermatogenesis are unpredictable, several testicular specimens may be required until sperm is found. The removal of large amounts of tissue may compromise the success of future retrieval attempts and is associated with transient or permanent adverse effects in the testis that may lead to hypogonadism.[8] Moreover, laboratory processing of such large quantities of testicular parenchyma is time-consuming and labor intensive.[9] The concept of micro-TESE is to identify the areas of sperm production within the testes with the aid of optical magnification, based on the size and appearance of the seminiferous tubules (ST).[10] It has been advocated that micro-TESE is superior to other methods of sperm acquisition, such as TESE and testicular sperm aspiration (TESA), yielding to greater success in obtaining sperm while minimizing tissue removal that ultimately facilitates sperm processing and alleviates testicular damage.[10-14] Given the fact that our center has in place a strict policy of not using gamete donation, and as our demand of azoospermic men with difficult case scenarios seeking fertility treatment has increased in recent years, we decided to implement micro-TESE as an alternative to TESE for sperm acquisition. The purpose of this study is to report our initial clinical and laboratory experience with microsurgical SR in a group of men with NOA and poor prognosis for SR. In addition, we describe the technical aspects of the microsurgical procedure for those considering implementing micro-TESE in their centers.

MATERIALS AND METHODS

Selection and description of participants

A cohort of 14 men aged 29-41 years (mean = 35 years) with NOA was enrolled in this study. The eligibility criteria were as follows: (i) Previous unsuccessful SR by either percutaneous TESA (n = 4) or conventional TESE (n = 3) or (ii) histopathology results from a diagnostic testicular biopsy revealing the presence of Sertoli-cell only (SCO) or maturation arrest (MA) (n = 6). A single patient with nonmosaic Klinefelter syndrome (KS) and severely hypotrophic testes (combined left + right testicular volume of 10 cc) who have not met the selection criteria, for neither a previous SR attempt nor a diagnostic testis biopsy had been performed prior to the micro-TESE, was included. A complete evaluation, including history, physical examination, and hormone profile, was available for all patients, as previously described.[15] Testicular volume was measured by physical examination using an orchidometer. Additionally, physical examination was used to detect or exclude the presence of a varicocele. Azoospermia was confirmed on at least two different centrifuged ejaculates according to World Health Organization guidelines.[16] Genetic screening for Yq microdeletions and G-band Giemsa Karyotype was obtained for all individuals, and results were normal but for one man in whom a nonmosaic 47,XXY karyotype was found. Hormonal evaluation including serum determination of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and total testosterone was obtained within 3-4 months prior to the micro-TESE attempt. Of the men, 11 (78.6%) had testosterone levels <300 ng/dL or testosterone (T) to estradiol (E) ratios (T in ng/dL, E in pg/mL) of <10 and were treated with medication to optimize endogenous testosterone production prior to micro-TESE.[17] Aromatase inhibitors (1 mg anastrozole daily), urinary-derived human chorionic gonadotrophin (urinary-derived hCG, 5000 IU subcutaneously every 4 days) or tamoxifen citrate (10 mg twice a day) were used for at least 2 months before micro-TESE. Patients were treated with aromatase inhibitor if T/E was <10 (n = 4), and with tamoxifen (n = 6) or hCG (n = 1) if T/E >10 with T < 300 ng/dL. When an adequate response (normalization of T/E ratio) was not achieved with the oral medical therapy in patients taking anastrozole, hCG injections or tamoxifen was added to the regimen (n = 3). None of our patients was taking exogenous testosterone therapy within at least 6 months prior to the time of therapy.

The patients and their female partners were given the option of either undergoing directly to micro-TESE concomitant to ovarian stimulation and oocyte retrieval, with cancellation of the sperm injection cycle if sperm were not retrieved, or having a trial micro-TESE with sperm cryopreservation and subsequent intracytoplasmic sperm injection (ICSI) attempt. All couples have chosen the former option.

Technical information

Microdissection testicular sperm extraction

All micro-TESEs were performed by the same team of urologists (SCE and DR) over a 7-day period, in May 2012, in the day prior to oocyte aspiration. Patients were asked to collect a semen specimen by masturbation immediately before the procedures to confirm azoospermia. In all cases, azoospermia was confirmed after analysis of centrifuged specimens. A senior urologist (SCE) with expertise in microsurgery was responsible to setup and implement the technique of micro-TESE as previously reported.[18] A list of surgical instruments and materials used for the procedures are presented in Table 1. Procedures were performed under epidural anesthesia with the patient positioned on an operating table in a supine position. A floor-standing operating microscope (OPMI Vario/S88 System, Karl Zeiss, Germany) was used throughout the procedures. After skin disinfecting and draping, the scrotal skin was stretched over the anterior surface of the testis, and a 2.5-cm transverse

incision was placed. The incision was carried out through the dartos muscle and tunica vaginalis. The tunica was opened and its bleeders cauterized. The testis was delivered extravaginally and the tunica albuginea was examined. A single large equatorial incision around approximately 270° of the circumference of the testis was made on an avascular area in the tunica albuginea under ×6-8 magnification, and the testicular parenchyma was widely exposed [Figure 1]. A testicular fragment of approximately 5 × 5 × 5 mm was excised from the medium testicular pole and placed in Bouin's fixative for histopathology examination. This amount of excised tissue yields a sufficient number of ST (>50 cross-sections) to perform an adequate quantitative analysis.[19] Dissection of the testicular parenchyma was undertaken at ×16-25 magnification searching for enlarged ST, which are more likely to contain germ cells, as originally described by Schlegel.[10] The superficial and deep testicular regions were examined, as needed, and microsurgical-guided testicular biopsies were performed by carefully removing enlarged and opaque tubules using microsurgical forceps [Figure 2]. If enlarged tubules were not seen, then two to three random micro-biopsies were performed at the upper, medium, and lower testicular poles. The excised specimens were placed into the center well of Petri dishes containing buffered sperm medium in room temperature.[20] Specimens were washed grossly to remove blood clots and sent to the IVF laboratory immersed in HEPES-buffered sperm medium kept at room temperature. The surgeon was promptly informed about the results of initial microscopic examination of each extracted specimen, which were delivered by the embryologist in approximately 3-5 minutes. This was possible since the

Table 1: List of instruments and materials used for micro-TESE operations

Microsurgical instruments
Straight nontoothed fine-tip forceps (13.5 cm long)
Curved nontoothed fine-tip forceps (13.5 cm long)
Nonlocking needle holder with a rounded finely curved tip
Pair of straight or curved-blunt dissecting scissors
Bipolar cautery with fine-tipped forceps
Blunt, long and rounded irrigating needle
Nonmicrosurgical instruments
Basic set of surgical instruments for small surgeries (including needle holder, smooth and toothed forceps (Addison forceps), suture scissors, curved dissection scissors, pair of Farabeuf retractors, scalpels for blades no. 11 and 23, curved Kelly clamps, straight mosquito clamps, Backhaus clamps)
20-cc syringes
1000-cc saline or lactate Ringer's solution
Unipolar cautery pen
Sutures (5-0 vycril with tapered needle, 5-0 catgut with tapered needle, 6-0 black monofilament nylon with taper cut needle)
Operating microscope configuration
Dual-headed binocular tube and eyepieces
200, 300, and 350-mm objective lens
Motorized foot-operated zoom system
Additional instruments and materials
Unipolar and bipolar coagulating generator
Antiseptic skin cleaning solution
Sterile towels
Gauze sponges
Sterile gowns
Surgical gloves
Surgical drapes
Surgical instrument table
IVF laboratory materials and reagents
50×9 mm and 60×15 mm Petri well dishes
Disposable serological pipettes
Pipettor 1-200 μL
6-mL sterile centrifuge polystyrene tubes with caps
0.7×25 mm needles and tuberculin syringes
HEPES-buffered sperm medium

TESE=Testicular sperm extraction; HEPES= (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid); IVF=*In vitro* fertilization

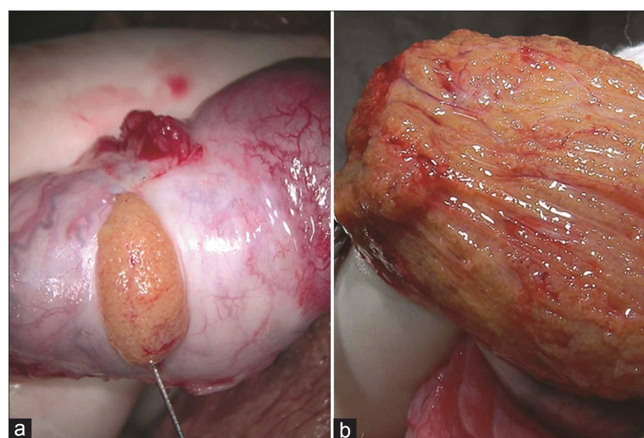

Figure 1: Microsurgical testicular sperm extraction. After testis exteriorization, a single and large incision is made in an avascular area of the albuginea (a) to expose the testicular parenchyma (b) with the aid of the operating microscope at ×6-8 magnification

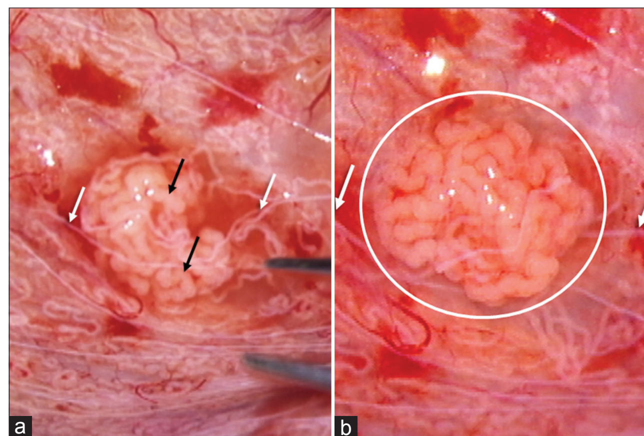

Figure 2: Identification of enlarged seminiferous tubules during micro-TESE: (a) Uniform distribution of collapsed tubules (white arrows) and the presence of one focus of clearly dilated tubules in the center (black arrows); (b) Dilated STs, highlighted in the center (circle), are surrounded by collapsed ones (white arrows). Photomicrographs were taken with the aid of the operating microscope at ×25 magnification

operating room and the IVF lab were located side-by-side with a communicating pass-through window. The albuginea was closed using continuous non-absorbable 6-0 nylon suture. Following hemostasis, the tunica vaginalis was closed in a running fashion using 5-0 absorbable suture. Then, the dartos muscle was closed with interrupted absorbable sutures. Last, the skin was closed with continuous subcuticular 5-0 Vycril suture, and a fluffy-type dressing and scrotal supporter were placed. The procedures were carried out at the contralateral testicle, as described earlier, when an insufficient number or no sperm have been found at initial laboratory examination. Patients were discharged 1 day after surgery. Prior to discharge, patients were examined to rule out scrotal hematoma. Bed rest and application of ice packing over the scrotum were recommended for the first 48 hours. Patients were instructed to remove the scrotal dressing after 24 hours, and were encouraged to take warm showers, and to wash the incision area with soap and water after 24 hours post-operatively. Oral analgesics for pain control were routinely used for 3 days. In cases of persistent pain, 50 mg tramadol bid was recommended. Additionally, oral nonsteroidal antiinflammatory was routinely used for 5 days. Patients were instructed to resume a normal diet and increase daily activities to a normal level over a 3 to 4 day period. The use of scrotal supporter was recommended for 10 days post-operatively. Patients were instructed to abstain from sports activities, heavy lifting, and sexual intercourse for 10 days, and were informed of the likelihood of scrotal swelling, ecchymosis at the wound site as well as mild discomfort that usually subside in approximately 1 week. All patients were advised to report any adverse signs and symptoms. These include fever, persistent pain and swelling, bleeding or excessive fluid leaking from the wound. Scrotal ultrasound was indicated in cases of complications.

Testicular specimen processing and sperm injections

Sterile handling conditions under a laminar flow cabinet were used during all laboratory steps. Micro-TESE fragments were handled under stereomicroscopy.[9,20] First, we used 23 gauge needled-tuberculin syringes to remove blood clots and disperse the STs.[20] Then, we transferred the specimens to dishes containing fresh sperm medium to be examined at the inverted microscope. The diameter of the tubules was determined using a digital imaging system (CIVA, Hamilton-Thorne, USA) attached to the inverted microscope. For this, the operator used the system to capture the images of individual ST at ×100 magnification. Measurements were taken in microns from edge to edge of the most dilated tubules, and the larger one from each patient was considered for analysis [Figure 3]. Subsequently, mechanical mincing of the STs was carried out using two needled-tuberculin syringes (one was used to hold the tubules in place at the bottom of the dish while the other squeezed and opened the tubules). We repeated this step until no intact tubules were seen. Homogenates were then examined on a warm staged inverted microscope at ×200-400 magnification to confirm the presence of sperm. If multiple micro-TESE specimens were received, all described steps were repeated. A minimum of two laboratory technicians/embryologists handled the micro-TESE specimens: One minced the tubules under stereomicroscopy and the other searched for spermatozoa under the inverted microscope. The surgeons were promptly informed when sperm were found on initial examination. When sperm were not observed after initial microscopic examination, extensive mechanical processing and searching were undertaken. For this, cell suspensions were diluted with sperm medium and centrifuged at ×300 g for 7 minutes. The supernatants were discharged and the pellets were resuspended in approximately 0.2 mL of sperm culture medium. Several petri dishes containing numbered microdroplets under mineral oil were prepared for sperm search and pick-up, and each microdroplet was loaded with approximately 1 μL of testicular cell suspension. Culture media temperature during sperm handling and processing was kept in the range of 32-37°C. Spermatozoa were identified and picked up for ICSI using the microinjection micropipette and transferred to a microdroplet of polyvynilpyrrolidone. Selected spermatozoa based on morphologic characteristics were immobilized, aspirated into the micropipette, and injected into the cytoplasm of metaphase-II oocytes. Sperm immobilization was carried out by firmly touching the tip of injection pipette to the transition zone between mid-piece and sperm tail.[21] The injected oocytes were transferred to a closed culture system and incubated for 16-18 hours at 37°C and 5.5% CO_2, until confirmation of fertilization. Fertilization

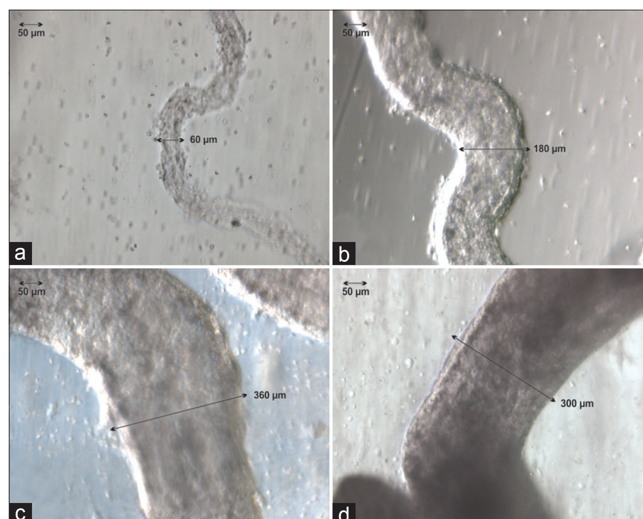

Figure 3: Measurements of seminiferous tubule diameter. A digital imaging system attached to the inverted microscope was used to capture the images of individual STs: (a) Collapsed ST extracted from a patient with failed sperm retrieval; (b) Noncollapsed ST extracted from a patient with failed SR whose testicular histology showed early maturation arrest; (c and d) Enlarged STs extracted from patients with successful SR

was considered normal when oocytes with two pronuclei were seen. Embryo cleavage was checked approximately 48 and 72 hours after ICSI and the number, symmetry, and expansion of the blastomeres, multinucleation, anomalies of the zona pellucida, and the rate of cytoplasmic fragmentation were recorded. The embryos were classified as top quality when they had three to four symmetrical blastomeres on the second day of culture and seven to eight symmetrical blastomeres on the third day, with no multi-nucleation, grade I (no fragmentation) or grade II fragmentation (up to 20% of the perivitelline space with fragments), and no abnormalities in the zona pellucida.[22]

Ovarian stimulation, oocyte retrieval, and embryo transfer

For all cycles, treatment was initiated with 0.25 mg daily subcutaneous administration of gonadotropin-releasing hormone agonist (GnRH-a; Buserelin acetate, Intas, India), starting on the 21st day of the menstrual cycle and was maintained until the day before human chorionic gonadotropin (hCG) administration. For ovarian stimulation, initial daily doses of 150-375 IU of recombinant human FSH (Gonal-F®, MerckSerono, India) were used. The initial dose of gonadotropin was determined by the treating physician taking into account female parameters such as age, body mass index, serum anti-Mullerian hormone (AMH) levels, serum FSH levels measured on day 2 or 3 of the menstrual cycle, baseline ovarian volume on transvaginal ultrasound (TVUS), and number of antral follicles seen on TVUS scan on days 2 or 3 of the menstrual cycle prior to ovarian stimulation. Ovarian stimulation commenced only after confirming pituitary down-regulation by both serum estradiol levels <50 pg/mL and the absence of ovarian follicles >10 mm in diameter on TVUS. Upon confirming down-regulation, the dose of GnRH analogue was reduced to 0.1 mg daily. Ultrasound assessment between the fifth and eighth days of stimulation was performed to determine if gonadotropin dose adjustments were required; if prevention of ovarian hyper-response was deemed necessary, the dose was reduced. There was no dose increase of gonadotropin during stimulation, even in cases of poor ovarian response. hCG was administered when two or more ovarian follicles reached a mean diameter of 17 mm. Recombinant choriogonadotropin 250 µg (Ovitrelle®, MerckSerono, India) was used for the final maturation of oocytes. Oocyte retrieval was performed under local anesthesia and guided by TVUS, 35 hours after hCG administration. After oocyte aspiration, follicular fluid was examined for cumulus-corona-oocyte complexes. The complexes were chemically denuded with 40 IU/mL of hyaluronidase (Sage, USA). The isolated oocytes were then mechanically denuded and classified according to nuclear maturity. The oocytes were maintained in culture until sperm microinjection. The fertilized oocytes were maintained in culture until transfer of the embryos to the uterine cavity guided by abdominal ultrasound on the third or fifth day of embryo culture.[23] Serum progesterone (P4) determination was routinely performed on the day of hCG administration. All embryos were electively cryopreserved when P4 levels were >1.5 ng/mL, due to the reported negative impact of such levels on implantation.[24] The cryotop vitrification method was used for embryo freezing.[25] Briefly, day 3 embryos were placed in an equilibration solution containing 7.5% ethylene glycol and 7.5% dimethyl sulfoxide (DMSO) in MOPS buffer for 5-8 minutes. Embryos were then moved to the vitrification solution containing 15% ethylene glycol and 15% DMSO and 0.6 M sucrose, and subsequently loaded onto cryotop and plunged in liquid nitrogen within 60 seconds. For thawing, cryotops were plunged into 1 M sucrose solution for 60 seconds (SAGE, USA). Embryos were then moved to 0.5 M sucrose solution for 3 minutes and transferred to culture media after equilibration and washing in MOPS buffer for 10 minutes.[25] Frozen-thawed embryo transfer was performed after 24-48 hours of culture.

Definitions and criteria

Success on micro-TESE was reported as the collection of any number of motile or immotile spermatozoa that allowed sperm injections to be performed. At least 50 ST cross-sections were evaluated on histopathology. Both the predominant and the most advanced stage of spermatogenesis were tabulated. SCO category indicated that tubules were lined with Sertoli cells and devoid of germ cells. MA was defined as absence of mature spermatozoa, despite normal early stages of spermatogenesis. Normal spermatogenesis was defined as the presence of tubules exhibiting all stages of the spermatogenesis up to mature sperm. Biochemical pregnancy was determined by measuring serum beta-hCG levels 15 days after egg retrieval. Clinical pregnancies were confirmed by a gestational sac with an embryo showing cardiac activity on ultrasound at weeks 6-7. Miscarriage was considered when nonviable clinical pregnancy was noted on ultrasound up to gestational week 20.

Data collection

Data was collected prospectively. Demographics and baseline characteristics of patient population, success of sperm acquisition, presence of motile sperm and surplus sperm for cryopreservation were obtained. The following outcomes were also obtained in the subgroup who have undergone sperm injections: Female demographics and baseline endocrine profile, number of aspirated oocytes, number of injected oocytes, rate of fertilization, number of top quality embryos assessed on day 3 of culture (per total number of embryos obtained), number of transferred/cryopreserved embryos, number of clinical pregnancies and miscarriages.

Ethics

Signed informed consent was obtained from every couple prior to enrollment into the micro-TESE and ICSI program,

including permission to use their data for analysis with guarantees of confidentiality. The study was exempted of institutional review board (IRB) approval since it involved the analysis of records from established clinical practices.

Statistics

Patient demographics and outcomes of sperm injection cycles were analyzed descriptively. Mann-Whitney U test and Fisher exact test were used to compare demographic parameters and baseline characteristics of men with successful and failed SRs. A $P<0.05$ was considered significant. All statistical data were processed with Statistica®, version 7.0.

RESULTS

The overall success of micro-TESE at obtaining testicular sperm was 50.0% (7/14). Micro-TESE was successful in 57.1% (4/7) of patients with a history of failed retrievals. Among the men who had unfavorable histology results from previous diagnostic testis biopsy, micro-TESE was successful in 50.0% (3/6). Testicular sperm was obtained in 50% of the retrieval attempts in the etiology categories of cryptorchidism (postorchidopexy), postorchitis, and idiopathic. Of the successful cases, retrieval of motile sperm was achieved in 5 (71.4%) cases. Among the men who had failed SRs, one had KS, one had a history of cryptorchidism and orchidopexy performed at age 20, and the remaining five had idiopathic NOA. Cryopreservation of surplus retrieved sperm was carried out in 3 (42.8%) cases. Patients' demographic characteristics, baseline endocrine profile, testicular volume, and the etiology of NOA are presented in Table 2.

The majority (64.3%) of the micro-TESE procedures was performed on both testes. Mean operative duration of unilateral and bilateral procedures were 84 (range 45-120) and 152 (range 120-195) minutes [Table 3]. Of these, SR was successful within the first 2 hours of operation in 5 (71.4%) cases. The mean number of testicular fragments excised was 10 (range 4-20).

Histopathology results of specimens obtained intraoperatively revealed the pattern of SCO in nine (64.3%) cases, MA in four (28.6%), and uniform hyalinization with rare foci of early MA in one case. We correlated the histopathology results with intraoperative findings, and noted that uniform tubule size and opacity was a common pattern of MA. In such cases, excised fragments were taken at random since no clearly distinction among tubules was possible using the operating microscope. Nevertheless, testicular sperm were retrieved in three of them. In cases of SCO, a clear microscopic distinction between collapsed and enlarged tubules was possible in five (55.5%) cases. Of them, testicular sperm were retrieved in four (80%) cases.

Spermatozoa were identified at initial laboratory screening in all but one case. In the latter, sperm was found after extended laboratory processing after a failed initial screening. The mean maximum diameter of individual excised ST was 224 microns (range: 90-350). No major complications occurred after micro-TESE procedures. Among the minor complications, pain was the most common complaint ($n=4$) and all patients had mild scrotal edema.

Table 2: Baseline characteristics of men with nonobstructive azoospermia who underwent micro-TESE

#	Age (years)	Etiology	Testicular volume (cc)		Presence of clinical varicocele	Baseline hormone levels				Postmedical therapy		Sperm retrieved	Presence of motile sperm	Surplus sperm frozen
			Left	Right		FSH (mIU/mL)	LH (mIU/mL)	Testosterone (ng/dL)	T/E ratio	Testosterone (ng/dL)	T/E ratio			
1	30	Systemic disease (renal failure)	12	12	No	12.9	9.2	592	13.4	NA	NA	Yes	Yes	No
2	31	Klinefelter syndrome	5	5	No	48.6	20.4	219	5.5	225	6.6	No	-	-
3	32	Cryptorchidism[1]	10	10	Yes	6.7	2.6	198	5.5	616	9.8	Yes	Yes	Yes
4	35	Idiopathic	15	15	Yes	3.3	1.5	300	5.9	517	10.2	Yes	No	No
5	39	Idiopathic	20	20	Yes	1.3	1.6	376	8.7	938	21.8	Yes	Yes	No
6	41	Idiopathic	8	8	No	17.6	7.7	371	4.5	571	11.4	No	-	-
7	37	Idiopathic	12	10	No	17.8	5.5	349	5.2	439	10.7	Yes	Yes	Yes
8	41	Idiopathic	20	20	No	5.0	4.2	226	6.6	424	9.2	No	-	-
9	40	Postorchitis	8	8	No	80.4	22.8	167	8.3	174	3.4	Yes	Yes	Yes
10	31	Idiopathic	12	12	No	5.4	1.4	450	8.2	720	39.6	Yes	No	No
11	30	Cryptorchidism[2]	8	6	No	20.8	6.1	383	7.2	443	8.1	No	-	-
12	34	Idiopathic	15	15	No	11.8	1.9	402	11.5	NA	NA	No	-	-
13	39	Idiopathic	15	15	Yes	18.2	4.6	714	11.5	NA	NA	No	-	-
14	34	Idiopathic	8	6	No	16.0	7.5	142	3.1	178	3.9	No	-	-

TESE=Testicular sperm extraction; NA=Non applicable, [1]orchidopexy at age 2, [2]orchidopexy at age 20. FSH=Follicle stimulating hormone; LH=luteinizing hormone: AMH=Anti-Mullerian hormone

Table 3: Operative findings and histopathology results of men with nonobstructive azoospermia who underwent micro-TESE

#	Testis side	Operative duration (min)	Intraoperative aspects		Maximum tubule diameter	Histopathology results	
			Main findings	Number of testicular fragments removed		Predominant pattern	Most advanced pattern
1	Unilateral	120	Uniform distribution of collapsed tubules; sparse foci of non collapsed and dilated opaque tubules	8	180	Sertoli-cell only	Focal areas of normal spermatogenesis
2	Bilateral	120	Diffuse replacement of seminiferous tubules by hyaline; sparse collapsed tubules	12	90	Hyalinization	Isolated tubules with maturation arrest at spermatogonia stage
3	Unilateral	75	Uniform distribution of collapsed tubules; sparse foci of clearly dilated tubules	4	320	Sertoli-cell only	Isolated tubules with maturation arrest at spermatogonia stage
4	Bilateral	180	Uniform distribution of collapsed tubules; sparse foci of non-collapsed and dilated opaque tubules	20	180	Sertoli-cell only	Isolated tubules with maturation arrest at spermatogonia stage
5	Unilateral	90	Uniform distribution of non-collapsed tubules with normal appearance	5	350	Maturation arrest at spermatogonia stage	Focal areas of normal spermatogenesis
6	Bilateral	135	Uniform distribution of collapsed tubules	12	130	Sertoli-cell only	Sertoli-cell only
7	Unilateral	45	Uniform distribution of non collapsed tubules with normal appearance	4	320	Maturation arrest at spermatogonia stage	Focal areas of normal spermatogenesis
8	Bilateral	195	Uniform distribution of non-collapsed tubules with normal appearance	12	240	Maturation arrest at primary spermatocyte stage	Maturation arrest at primary spermatocyte stage
9	Unilateral	90	Diffuse homogeneous pattern of collapsed tubules; sparse foci of dilated tubules	4	240	Sertoli-cell only	Sertoli-cell only
10	Bilateral	180	Uniform distribution of non-collapsed tubules with normal appearance	16	240	Maturation arrest at spermatogonia stage	Maturation arrest at spermatogonia stage
11	Bilateral	135	Uniform distribution of collapsed tubules	12	150	Sertoli-cell only	Sertoli-cell only
12	Bilateral	180	Uniform distribution of collapsed tubules; foci of non collapsed opaque tubules with normal appearance	12	220	Sertoli-cell only	Focal areas of maturation arrest at spermatogonia stage
13	Bilateral	120	Uniform distribution of collapsed tubules	12	220	Sertoli-cell only	Sertoli-cell only
14	Bilateral	120	Uniform distribution of collapsed tubules; intense replacement of seminiferous tubules by hyaline	12	200	Sertoli-cell only	Sertoli-cell only

TESE=Testicular sperm extraction

Of the 11 (78.6%) men who received medical therapy before micro-TESE, 5 (45.5%) had initial testosterone levels <300 ng/dL and 6 (54.5%) had T/E ratio <10. Among the treated individuals, eight (73%) responded and had either serum testosterone levels >300 ng/dL or T/E ratios >10 before micro-TESE, while three (17%) did not. Sperm retrieval rates (SRRs) did not differ between men who responded or not to medical therapy (83% vs. 33%; *P*=0.54, two-tailed Fisher exact), and between those who received medication or not (54% vs. 33%; *P*=1.0, two-tailed Fisher exact). There were no differences between patients with successful and failed micro-TESE with respect to the proportion of medical therapy administration, posttherapy testosterone levels, presence of varicocele, testicular histology categories, and male baseline characteristics [Table 4]. Fewer men with successful retrievals underwent bilateral micro-TESE procedures (28.6%) in comparison to the failed group (100%; *P*=0.02). The average maximum seminiferous tubule diameter was higher in the group with successful (261.4 ± 69.4 microns) compared with failed retrievals (178.6 ± 55.8 microns; *P*=0.04).

Sperm injections were carried out for seven couples using testicular sperm retrieved by micro-TESE [Table 5].

Table 4: Demographics and operative characteristics of patients with successful and failed micro-TESE

	Successful micro-TESE (*n*=7)	Failed micro-TESE (*n*=7)	*P* value
Mean±SD (95% CI) male age (years)	34.8±3.9 (30; 40)	35.7±4.6 (30; 41)	0.70 (Mann-Whitney)
Mean±SD (95% CI) testicular volume† (cc)	25.1±7.8 (16; 40)	22.0±12.2 (10; 40)	0.45 (Mann-Whitney)
Mean±SD (95% CI) baseline hormone levels			
FSH (mIU/mL)	18.2±27.9 (1.3; 80.4)	19.7±13.7 (5.0; 48.6)	0.25 (Mann-Whitney)
LH (mUI/mL)	6.4±7.8 (1.4; 22.8)	7.5±6.0 (1.9; 20.4)	0.38 (Mann-Whitney)
Total testosterone (ng/dL)	347.4±146.2 (167.0; 592.0)	351.0±188.2 (142.0; 714.0)	0.76 (Mann-Whitney)
T/E ratio	7.9±2.8 (5.2; 13.4)	7.1±3.3 (3.1; 11.5)	0.53 (Mann-Whitney)
Mean±SD (95% CI) operative time (min)	111.4±51.8 (45.0; 180.0)	143.6±31.0 (120.0; 195.0)	0.12 (Mann-Whitney)
No. Varicocele (%)	3 (42.8)	1 (14.3)	0.55 (Fisher exact)
No. Medical therapy before micro-TESE	6 (85.7)	5 (71.4)	0.12 (Mann-Whitney)
Mean±SD (95% CI) pretreatment T/E ratio	6.9±1.6 (5.2; 8.7)	5.4±1.6 (3.1; 7.2)	0.16 (Mann-Whitney)
Mean±SD (95% CI) posttreatment T/E ratio	15.9±13.0 (3.4; 39.6)	7.8±2.8 (3.9; 11.4)	0.20 (Mann-Whitney)
Mean±SD (95% CI) posttherapy testosterone (ng/dL)	567.3±249.5 (174.0; 938.0)	368.2±163.2 (178.0; 571.0)	0.20 (Mann-Whitney)
No. Bilateral retrievals (%)	2 (28.6)	7/7 (100.0)	0.02 (Fisher exact)
No. Predominant histology (%)			
Sertoli-cell only	4 (57.1)	6 (86.0)	0.55 (Fisher exact)
No. Maturation Arrest	3 (42.9)	1 (14.0)	0.55 (Fisher exact)
Mean±SD (95% CI) seminiferous tubule diameter	261.4±69.4 (180.0; 350.)	178.6±55.8 (90.0; 240.0)	0.04 (Mann-Whitney)
Mean±SD (95% CI) No. Fragments Removed	8.7±6.6 (4−20)	12±0.0 (12;12)	0.20 (Mann-Whitney)

CI=Confidence interval; TESE=Testicular sperm extraction; FSH=Follicle stimulating hormone; LH=luteinizing hormone: AMH=Anti-Mullerian hormone

Table 5: Baseline characteristics of female partners and sperm injection outcomes of successful micro-TESE cases

No. Sperm injection cycles	7
Mean±SD (95% CI) female age (years)	30.6±5.8 (25.0; 39)
Mean±SD (95% CI) infertility duration (years)	6.3±2.3 (3.0; 9.0)
Mean±SD (95% CI) baseline hormone levels	
FSH (mIU/mL)	6.1±1.7 (3.6; 8.0)
LH (mUI/mL)	4.1±2.5 (0.8; 7.2)
AMH (ng/mL)	1.8±1.4 (0.3; 3.8)
No. Associated female infertility (%)	3 (42.8)
Gonadotropin dose for ovarian stimulation (UI)	2025.0±876.8 (975.0; 3325.0)
Mean±SD (95% CI) oocytes (%)	
Retrieved	10.0±5.0 (3.0; 16.0)
Injected	7.1±3.9 (3.0; 14.0)
Fertilized (2PN)	4.6±3.5 (1.0; 11.0)
No. Embryos (%)	
Cleaved	3.4±3.9 (0.0; 11.0)
Transferred fresh	1.5±0.6 (1.0; 2.0)
Transferred frozen-thawed	1.5±0.0 (1.0; 2.0)
Frozen	3.0±3.1 (0.0; 8.0)
No. Clinical/ongoing pregnancies (%)	
Per initiated cycle with sperm injection	2 (28.6)
Per transfer*	2 (40.0)

*Considering five fresh+frozen-thawed embryo transfers; FSH=Follicle stimulating hormone; LH=luteinizing hormone: AMH=Anti-Mullerian hormone

Injections were performed using motile sperm in six (85.7%) cases. In the remaining, injections were carried out using the hyposmotic swelling test to assess sperm vitality.[9,20] Normal two pronuclei fertilization rate after sperm injections was 64%. The cleavage rate of fertilized zygotes was 75%. Two fresh transfers were performed with an average of 1.5 ± 0.6 embryos replaced to the uterine cavity. Of these, none resulted in pregnancies. One cycle was cancelled due to abnormal fertilization, whereas four fresh transfers were cancelled due to elevated serum progesterone levels on the day of hCG administration. A total of 15 embryos were cryopreserved. Of these, 11 were from patients who had their fresh transfers cancelled due to elevated serum progesterone levels. Three frozen-thawed embryo transfers were carried out with an average of 1.5 (range 1-2) embryos, and two of them resulted in clinical pregnancies that are currently ongoing. Cumulative clinical pregnancy rate per initiated cycle with sperm injection and embryo transfer was 28.6%, with an implantation rate of 33.3% considering fresh and frozen-thawed transfers. Among the failed SR cases, the retrieved oocytes were either vitrified or discarded depending upon the couple's preference.

DISCUSSION

Micro-TESE was a reliable method to obtain sperm for ICSI in our group of men with NOA and poor prognosis for SR.

A cumulative SRR of 50% was obtained and sperm injections using the retrieved testicular sperm were carried out for all successful cases. Normal fertilization after sperm injections was achieved in 64% of the oocytes and morphologically normal cleaved embryos for transfer were available for all but one couple. Among the initiated cycles with successful SR and embryo transfers, an ongoing pregnancy rate of 28.6% was obtained in this series.

Our study depended primarily on the use of micro-TESE in men with NOA who either failed previous SR attempts or had unfavorable histopathology results on previous diagnostic testis biopsy. NOA is an untreatable condition associated with testicular failure that comprises a spectrum of testicular histopathology resulting from various causes that include genetic and congenital abnormalities, postinfectious, exposure to gonadotoxins, trauma, endocrine disorders, and idiopathic.[15] The only option for men with NOA to achieve biological fatherhood is to attempt SR and ICSI. Given the fact that our center's policy mandates the use of the couple's own genetic material during ART, we opted to implement the microsurgical method for SR due to the reported higher effectiveness of this procedure to retrieve testicular sperm in NOA. Retrieval rates ranging from 35% to 77% have been reported for micro-TESE,[2,3,6,10,17] and more importantly, controlled series demonstrated that micro-TESE performed better than conventional sperm extraction (TESE) or percutaneous aspirations (TESA).[11-14,26] Nonetheless, the adoption of strict criteria to diagnose NOA is crucial for the indication of micro-TESE since it is an invasive procedure with potential complications.[2,3,27] Hence, we confirmed the diagnosis of NOA by histologic evaluation in all individuals. In addition, we confirmed the presence of azoospermia by analyzing centrifuged semen specimens obtained immediately before the procedures since rare sperm may occasionally spill over into the ejaculates of such patients.[2] Studies focusing on quantitative spermatogenesis have shown that the threshold of three mature spermatids per seminiferous tubule's cross-section must be exceeded in order for spermatozoa to spill over into the ejaculate. Men with NOA have a mean of 0-3 mature spermatids per seminiferous tubule, thus explaining why rare sperms are occasionally found in their ejaculates.[2,28]

Success at obtaining testicular sperm by micro-TESE has shown to be comparable among different etiology categories of cryptorchidism, varicocele, orchitis, genetic, radio-/chemotherapy, and idiopathic.[18,29-31] Furthermore, micro-TESE has shown to be successful in approximately one-third of previous failed retrievals by other methods.[9,10] Here, we were successful in 50% of our retrieval attempts in the etiology categories of cryptorchidism, postorchitis and idiopathic, and in two-third of cases with a history of previous failed retrievals using non-microsurgical retrieval methods. Furthermore, surplus sperm for freezing were available in nearly half of the men with successful retrievals. This is advantageous since cryopreservation may prevent the need for future retrievals in case ICSI fails. However, studies have reported conflicting findings with regard to the reproductive potential of fresh and frozen-thawed testicular sperm from men with NOA.[32-37] While some investigators have suggested impaired fertilization,[33] embryo development,[34] and implantation[35] using frozen testicular sperm compared with fresh, others could not find significant differences in these parameters.[36,37] In a meta-analysis of 10 studies involving 825 cycles, fertilization rates remained similar but implantation rates were significantly higher (by 73%) when ICSI was performed with fresh rather than frozen-thawed testicular sperm.[35] However, ongoing pregnancy rates in the aforementioned study were not negatively affected by the state of testicular sperm used for ICSI, that is, fresh or frozen-thawed (relative risk [RR]: 0.88; 95% confidence interval [CI]: 0.58-1.33). Despite this controversy, repeated micro-TESE, if needed, may result in successful SR of approximately 82% enabling ICSI to be performed with fresh sperm.[38] In such cases, it is advisable to allow an interval of 6 months between retrievals as repeated procedures are more likely to be successful after 6 months compared with those performed within 6 months (8% vs. 25%).[8] We failed to retrieve sperm in five men with idiopathic NOA, one man with history of cryptorchidism and orchidopexy performed at age 20, and one man with KS. The age at orchidopexy seems to impact sperm recovery, as shown in a study by Vernaeve *et al*., who noted that the mean age at orchidopexy significantly differed in men with positive (10.6 years) and negative (15.5 years) sperm recovery.[39] Despite being unable to obtain sperm in our patient with KS, SRR of micro-TESE in such men have been reported to range from 55% to 75%.[17,27]

The options for SR in difficult cases of NOA are either the removal of larger amounts of tissues[2] or microsurgical identification of morphologically dilated STs that are more likely to harbor sperm production, as originally described by Schlegel.[10] While we were successful overall in our retrieval attempts with only minor postoperative complications, micro-TESE has advantages and disadvantages compared with other open surgical methods. Implementation of micro-TESE in a clinical ART setting requires the availability of an operating room equipped with a top-quality operating microscope located next door to the IVF laboratory. Moreover, micro-TESE is a labor-intensive procedure that requires microsurgical expertise. In our series, the mean operative time was approximately 2 hours; however, operations ranged from 45 minutes to more than 3 hours. We were successful in retrieving sperm within the first 2 hours in 71.4% of cases. Furthermore, spermatozoa was identified at initial laboratory screening in all but one case

that required extended laboratory processing to find sperm after a failed initial screening. Our results are corroborated by a recent series in which the best chance of sperm recovery during micro TESE occurred within the first 2 hours of the operation. However, in up to 37% of operations in the aforementioned study more than 4 hours were required to achieve success.[40] All these together add to the cost of IVF and limit the widespread applicability of micro-TESE.[18] In contrast, the use of operating microscope during TESE limits the risk of vascular injury and optimizes the chances of finding sperm. Proper identification of testicular vessels under the tunica albuginea is made prior to the placement of an incision into the testis. Microsurgery also allows preservation of intratesticular blood supply; excellent hemostasis is achieved, thus reducing the chance of complications due to hematoma formation and testicular devascularization as it may occur in cases of conventional TESE.[2,12,27] In addition, removal of excessive amount of testicular parenchyma may jeopardize androgen production and limit the chances of repeated SR, which are particularly important for men with NOA who usually have small and highly dysfunctional testes.[8] Despite declining after micro-TESE, androgen production recovers to preoperative levels in the vast majority of patients within 12−18 months. However, men with KS should be monitored closely for hypogonadism since androgen production is restored to only 50-75% of preoperative values after micro-TESE.[41,42]

From the laboratory standpoint, the small amount of tissue extracted by micro-TESE facilitates sperm processing. This is advantageous for processing of TESE specimens may be incredibly labor-intensive and the searching process may miss the rare spermatozoa within a sea of STs and other cells. [18,40] It has been reported that the optimal fertilization by ICSI using surgically retrieved sperm is obtained when the time frame from hCG administration to microinjection does not exceed 44 hours.[43] As such, operations and laboratory processing of extracted specimens should be coupled with oocyte aspiration and sperm injections in a coordinated manner. Selection of spermatozoa from a smaller population of contaminating testicular cells facilitates the laboratory steps of this complex process.[20] In this present series, we confirmed that it was far less technically demanding and labor-intensive to extract spermatozoa from small volume specimens than large pieces of testicular tissue that must be dissected, red blood cells lysed, and the rare spermatozoa searched for in a tedious fashion under an inverted microscope. We experienced more ease for sperm search, less contamination and blockage of the microinjection needle with cells and debris that resulted in greater speed for sperm pick-up and injections.

To date, there are still no absolute preoperative predictive factors for successful SR in NOA. Follicle-stimulating hormone, testosterone levels and testes volume reflect global testicular function and not the presence of a site of normal sperm production within a dysfunctional testis.[44,45] Testicular histopathology results, in contrast, confer better prognostic value compared with the aforesaid markers. SRRs by micro-TESE are significantly higher in hypospermatogenesis (93%) compared with MA (64%) and SCO (20%).[26] Nonetheless, successful retrievals are reported even in the more adverse histopathology pattern of SCO, as shown in the aforementioned study. This observation indicates that sperm production is distributed in a heterogeneous pattern within the testis and histologic assessment of a single testicular fragment is limited in its ability to determine the presence of rare foci of sperm production in NOA.[2,26] In our series, preoperative levels of FSH and testosterone, testis volume, and the proportion of patients with MA or SCO on specimens taken during micro-TESE did not differ in men with failed or successful retrievals. We were able to retrieve testicular sperm in approximately half of the men in whom the predominant histopathology pattern was SCO or MA. Interestingly, sparse STs containing foci of germ cells were observed in SCO cases, thus reaffirming the heterogeneity of sperm within the testis. In our series, the diameter of the excised STs was the only parameter associated with the chance of obtaining sperm. Notably, a clear distinction between collapsed and enlarged tubules was possible in one-third of our cases and sperm were retrieved for all but one patient. Conversely, the presence of a uniform pattern of collapsed tubules with none different in size was associated with failed retrievals in all cases. Histologic examination revealed that such cases were SCO. Along the same lines, we were not able to make a distinction on which tubules to extract in cases where all tubules were uniformly normal in size. Histologic evaluation revealed that such cases were classified as MA in which ST contained germ cell and were therefore normal in size. Hence, it was impossible to distinguish the ones with full maturation to spermatozoa, if any, by using the operating microscope.[2] Our results showing greater tubule diameter in cases of successful retrievals are corroborated by a recent report in which the mean maximal diameter of sperm-containing ST was significantly higher than nonsperm containing tubules (298 vs. 225 microns, $P < 0.0001$).[46] The best sensitivity and specificity for a positive result on SR was obtained at a cut-off level of 250 microns.[46] Our findings highlight the usefulness and limitations of the operating microscope to identify sperm-containing ST. Current research is focusing on novel tools that can aid in the identification of sperm-producing tubules without the need of tissue removal. Multiphoton microscopy has been applied successfully to differentiate normal from abnormal spermatogenesis in an *ex vivo* rodent model, and encouraging preliminary results have been reported in humans.[47,48] Confocal fluorescence microscopy has also

been used in a murine model of microdissection TESE.[49] Although the latter offers the advantage of rapid *in vivo* detection of sperm in the ST, the use of fluorescein to label sperm may limit the translation of this method to the clinical setting. Last, full field optical coherence tomography, which uses a safe light source with apparent no detrimental effect on sperm quality, was recently described as a useful tool to facilitate real-time visualization of spermatogenesis in an *ex vivo* rodent SCO model.[50] The aforesaid methods have the potential to be coupled with the operating microscope and aid in micro-TESE.

Medical therapy to enhance testosterone production prior to SR has been suggested to optimize retrieval rates in men with NOA.[1,17] The rationale of such intervention relies on the fact that most men with NOA have testes of reduced volume, which is associated with decreased testosterone production and hypogonadism. Adequate levels of intratesticular androgenic bioactivity are essential to sustain spermatogenesis that might be compromised in NOA.[51] Indeed, aromatase inhibitors, clomiphene citrate and hCG have been successfully used to boost testosterone production in men with NOA and nonmosaic KS. It has been shown that SR rates were increased by 1.4-fold in KS men who responded to medical therapy.[17] Nearly 80% of men in our series had signs of hypogonadism, as determined by either low serum testosterone or abnormal testosterone to estradiol ratios, and received medical therapy prior to micro-TESE. Despite being higher in men who responded to medical therapy (83% vs. 33%), SRRs did not attain statistical significance. Moreover, the proportion of men with successful and failed retrievals who received medical therapy, as well as their pre- and posttreatment endocrine profile, was not different. Nevertheless, these findings should be interpreted with caution since our sample population is too small to draw valid conclusions. Despite being greatly anticipated in men with NOA who will be halted in their attempt to conceive due to absence of testicular sperm on retrieval, medical treatment is still under investigation.[51] In a recent retrospective study on the role of optimizing testosterone before micro-TESE in men with NOA, Reifsnyder *et al*. evaluated 736 individuals and concluded that hormonal therapy had no impact on retrieval rate.[1] As such, a definitive conclusion cannot yet be drawn on the role of medical therapy in NOA until randomized trials including different subsets of men with NOA in whom intratesticular androgenic activity is measured can solve this dilemma.

After retrieval of testicular sperm from men with NOA, ICSI is used to bypass this severe male infertility condition.[3] In our series, sperm injections were performed with fresh testicular sperm in seven cases. Fertilization and embryo development after ICSI were in agreement with those reported in the literature,[1,3,9,29-32] and an ongoing cumulative pregnancy rate per transfer of 28.5% was reassuring. Indeed, the literature is rich in studies focusing at sperm injection outcomes after sperm acquisition. Fertilization and implantation rates are lower in NOA compared with obstructive azoospermia and ejaculated sperm.[3,9] Clinical pregnancy and live birth rates are also lower in NOA than other male infertility scenarios.[3,9,52,53] In one report comparing the reproductive potential of azoospermic men undergoing SR and ICSI, the likelihood of obtaining a live birth was 1.8-fold higher (odds ratio [OR] = 1.86; 95% CI: 1.03-2.89) in men with OA compared with men with NOA.[52] These findings indicate that men with NOA undergoing ART have decreased reproductive potential, which may be explained by a higher tendency of their testicular sperm to carry deficiencies related to the centrioles and genetic material. As a result, the ability of the male gamete to activate the egg and trigger formation and development of a normal zygote and a viable embryo is impaired.[53] Nevertheless, the neonatal profile of babies born seems not to be affected by the type of azoospermia or the source of sperm used for ICSI. Malformation and perinatal death rates in NOA range from 1.3% to 5.2% in large cohorts.[54,55] However, given the fact that the currently available data is based on a very limited population of children born, a call for continuing monitoring is warranted.

To the best of our knowledge, our study is the first to report the implementation and outcomes of micro-TESE in India. The main limitation of our study is the small patient cohort and the few data on sperm injection outcomes. Despite of that, we add to the existing literature by providing technical details of how we successfully implemented micro-TESE in our ART settings, and by reporting our initial experience with micro-TESE and ICSI. Our initial results with micro-TESE are reassuring and the information provided can be useful for doctors treating male infertility who wish to improve the chances of SR during TESE. In addition, our histopathology data can be used as a counseling tool for doctors dealing with men with NOA seeking fertility advice as for the presence of SCO is not an indicator of absolute sterility. A future direction would be the collection of additional data including the obstetric and short-term neonatal profile of babies born from such fathers.

CONCLUSIONS

The goals of SR are to obtain the best quality sperm possible in adequate numbers for immediate use and/or cryopreservation while minimizing the damage to the reproductive tract. In NOA, sperm production can be either markedly impaired or absent. As such, open surgical testicular SR is recommended to optimize the chances of finding sperm. Our data reaffirm the existing knowledge

that micro-TESE provides success in approximately 50% of men with NOA. Our initial experience with micro-TESE was associated with minor complications, and with marked reduction in time processing of testicular specimens. We were successful in implementing the technique to our andrology department, and in integrating the laboratory steps to our current embryology laboratory. Micro-TESE has shown to be a promising option to retrieve spermatozoa from the most severe cases of NOA.

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