

Microdissection Testicular Sperm Extraction (Micro-TESE): Results of a Large Series from India

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Abstract

Objective: We describe our micro-TESE experience in a large group of men with Non-Obstructive Azoospermia (NOA) and poor prognosis for Sperm Retrieval (SR), and critically analyze the method's results and limitations.

Methods: An ART facility was setup in a tertiary care center to perform SR using microsurgery. One hundred and eighty men with NOA underwent micro-TESE while their female partners received ovarian stimulation for Oocyte Pickup (OCP). Micro-TESE was performed on the day prior to OCP, and surgically-retrieved testicular sperm were used for sperm injections. We assessed sperm retrieval rates, operative aspects, and ICSI outcomes.

Results: The success of micro-TESE at obtaining testicular sperm for Intracytoplasmic Sperm Injection (ICSI) was 54.4% with no major complications. Sperm were obtained in 73.6 % of cases in which clearly dilated seminiferous tubules were seen, with minimal tissue excision which facilitated laboratory processing. Patients with successful and failed retrievals did not differ with respect to baseline characteristics, and presence of varicocele. Retrieval rates differed pertaining to testicular histology category. Also, retrieval rates were higher (53.1% vs. 35.6%) in patients who received medication to boost testosterone production prior to micro-TESE compared with those who did not. Sperm injections resulted in normal fertilization and embryo cleavage of 61% and 75%, respectively. A cumulative clinical pregnancy rate per ICSI cycle of 29.78 %, with an implantation rate of 19 % was achieved.

Conclusions: Micro-TESE is a valid method of SR in NOA. It yields sustainable results in poor prognosis azoospermic patients, with minimal damage to the testes. Our experience with micro-TESE applied to the most difficult cases of azoospermia is very reassuring, and we advocate that micro-TESE should be the method of choice in such cases.

Keywords: Sperm retrieval techniques; Azoospermia; Assisted reproductive techniques; Male infertility; Testis; Spermatozoa; Microsurgery; Sterility

Abbreviations: NOA: Non Obstructive Azoospermia; OA: Obstructive Azoospermia; TESE: Testicular Sperm Extraction; SR: Sperm Retrieval; SSR: Surgical Sperm Retrieval; PESA: Percutaneous Sperm Aspiration; TESA: Testicular Sperm Aspiration; IVF: In Vitro Fertilization; ICSI: Intra Cytoplasmic Sperm Injection

Introduction

Microdissection Testicular Sperm Extraction (micro-TESE) is a known surgical method of surgical Sperm Retrieval (SR) for men with Non-Obstructive Azoospermia (NOA) seeking fertility. Though they have highly dysfunctional testis often suggestive of testicular failure, rare foci of sperm production may exist in up to 60% of these individuals [1,2]. Sperm production, if present, is insufficient 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 with the aim of finding viable testicular sperm to be used for *in vitro* fertilization [3].

Historically, the method of choice to retrieve sperm in NOA has been Testicular Sperm Extraction (TESE), with success varying from 25% to 60% [3-7]. In TESE, open single or multiple testicular biopsies are randomly taken, processed and examined for the presence of sperm. Since prediction of both the existence and the geographic location of islets of normal spermatogenesis are not possible, more than one specimen may be required until sperm is found. In NOA, removal of large fragments of testicular tissue usually compromise androgen production, which can be transient or permanent ultimately resulting in

hypogonadism [8]. Also, laboratory processing of such large quantities of testicular tissue is time-consuming and labor intensive [9]. The concept of micro-TESE is to identify the areas of probable sperm production within the testes based on the size and appearance of the seminiferous tubules, with the aid of optical magnification [10]. Micro-TESE is advocated to be more efficient to other methods of sperm acquisition, such as TESE and Testicular Sperm Aspiration (TESA). The reasons are the greater success in obtaining sperm and lower tissue removal that facilitates sperm processing and lessens testicular damage [10-14]. As our patient population comprises mostly of couples for whom gamete donation is not acceptable, and as the demand of azoospermic men with difficult cases scenarios seeking fertility treatment has increased in our center in recent years, we decided to implement micro-TESE as an alternative to TESE for sperm retrieval in such patients [15].

Due to the promising results of micro-TESE reported in our preliminary series, we decided to continue applying this method of sperm acquisition to our patients with NOA.

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The purpose of this study is to report a two-year clinical and laboratory experience of microsurgical sperm retrieval in a large group of men with NOA and poor prognosis for sperm retrieval.

Methods

Selection and description of participants

A cohort of 180 men aged 29 to 41 years (mean=35 years) with non-obstructive azoospermia who underwent micro-TESE between May 2012 and November 2013 were studied. All men underwent complete evaluation, including history, physical examination and hormone profile as previously described by Esteves et al. [16]. Physical examination was used to diagnose or exclude the presence of a varicocele. Azoospermia was confirmed for all the patients on at least two different occasions by testing the centrifuged ejaculates according to World Health Organization guidelines [17]. G-band Giemsa Karyotype was obtained for all individuals, and results were normal but for 3 men in whom a non-mosaic 47, XXY karyotype were found. Genetic screening for Yq microdeletions was also performed. The eligibility criteria for micro-TESE were at least one of the following: i) previous unsuccessful sperm retrieval by either percutaneous testicular sperm aspiration or conventional testicular sperm extraction, ii) histopathology results from a previous diagnostic testicular biopsy revealing the presence of Sertoli cell-only or maturation arrest, iii) Klinefelter syndrome; iv) Y chromosome microdeletion involving the subregion AZFc. Hormonal evaluation including serum determination of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), estradiol and total testosterone was obtained within 1-2 months prior to the micro-TESE attempt. Of 180 men, 79 (43.8%) had testosterone levels <300 ng/dL or Testosterone (T) to Estradiol (E) ratio (testosterone in ng/dL, estradiol in pg/mL) of <10, and were treated with medication to optimize endogenous testosterone levels prior to micro-TESE [18]. Aromatase inhibitors (1 mg anastrozole daily), urinary human chorionic gonadotrophin (urinary-derived hCG, 5000 IU subcutaneously every 4 days) or tamoxifen citrate (10 mg twice a day) were used either as a single agent or in combination for at least 2 months before micro-TESE. Patients were treated with aromatase inhibitor if T/E was <10 (n=31), and with tamoxifen (n=35) or hCG (n=10) 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 or tamoxifen, hCG injections was added to the regimen (n=3). None of our patients took 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 going directly for 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 ICSI attempt. All couples chose the former option.

For all the men, a post centrifuge semen sample was evaluated on the morning of the micro-TESE procedure for presence of sperms. In all cases, assessment of sperm pellet revealed no spermatozoa.

Technical information

Microdissection testicular sperm extraction (micro-TESE): All micro-TESEs were performed by the same urologist (DR) on the day prior to oocyte aspiration. A Senior Urologist (SE) with expertise in microsurgery was responsible to setup and implement the technique of micro-TESE, as well as to provide training for the consultant urologist, as

previously reported [19]. Briefly, the 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, India) was used throughout the operations. After adequate skin disinfecting and draping, the scrotal skin was stretched over the anterior surface of the testis, and a 2.5-cms transverse incision was placed. This incision was carried out through the dartos muscle and tunica vaginalis. The tunica was opened and its bleeders were cauterized. The testis was delivered extravaginally and the tunica albuginea was examined to select the site of injection. A single large equatorial incision covering 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. A tiny testicular fragment of approximately 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 seminiferous tubules (>20 cross-sections) to perform an adequate quantitative analysis of the specimen [20]. Dissection of the testicular parenchyma was undertaken at 16-25 magnification in search for enlarged seminiferous tubules which are more likely to contain germ cells [10]. The superficial and deep testicular regions were examined, as needed, and operating microscope-guided testicular biopsies were performed by carefully removing enlarged and opaque seminiferous tubules using microsurgical forceps (Figure 1). If enlarged tubules were not seen after thorough inspection, then two to three random micro-biopsies were performed at the upper, medium and lower testicular poles. The excised biopsy specimens were placed into the center well of Petri dishes containing buffered sperm medium. Specimens were then washed to remove blood clots and sent to the IVF laboratory for processing and searching for sperm. Closure of albuginea was done using continuous non-absorbable 6-0 nylon suture. Following adequate hemostasis, the tunica vaginalis was closed in a running fashion using 5-0 absorbable suture. Dartos muscle was closed using interrupted absorbable sutures. Lastly, the skin was closed using continuous subcuticular 5-0 Vicryl suture, and a fluffy-type dressing and scrotal supporter were placed. The procedure was carried out at the contralateral testicle, as described above; when an insufficient number or no sperm have been found at initial laboratory examination on the first side. Patients were discharged 1 day post-surgery after examining them to rule out scrotal hematoma. Bed rest and ice pack application over the scrotum were recommended for the first 48 hours.

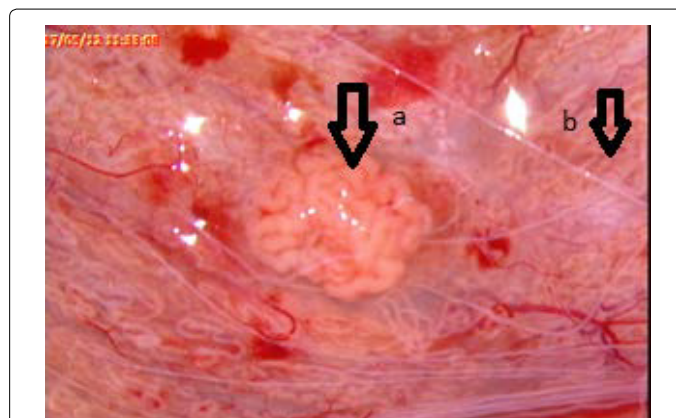


Figure 1: Testicular tissue with aid of operating microscope at x25 magnification
a: Dilated foci of seminiferous tubule
b: Surrounding collapsed seminiferous tubules

Patients were advised 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 postoperatively. Oral analgesics for pain control were routinely prescribed for 3 days. In cases of persistent pain, 50 mg tramadol bid was added. Patients were advised 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 continued for 10 days postoperatively. Patients were advised 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 subsides in approximately 1 week. All patients were advised to report any adverse signs and symptoms post operatively. These include fever, persistent pain and swelling, bleeding or excessive fluid leakage from the wound. Scrotal ultrasound was indicated in cases of any such complications.

Testicular specimen processing and sperm injection: All laboratory procedures were performed in sterile handling conditions under a laminar flow cabinet. Micro-TESE tissue fragments were handled under stereomicroscopy, as previously described [9,21]. To begin with, 23 gauge needled-tuberculin syringes were used to remove blood clots and disperse the Seminiferous Tubules (ST). Then, specimens were transferred to the dishes containing fresh sperm medium to be examined under the inverted microscope. A digital imaging system attached to the inverted microscope (CIVA, Hamilton-Thorne, USA) was utilized to measure the diameter of the tubules. For this, the images of individual seminiferous tubules were captured at x100 magnification. Measurements were taken in microns from edge to edge of the most dilated tubules, and the largest one from each patient was considered for analysis. Subsequently, mechanical mincing of the seminiferous tubules 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 to squeeze open the tubules). This process was repeated until no intact tubules were seen. Homogenates were then examined on a warm staged inverted microscope at x200-400 magnification to look for the presence of sperm. If multiple micro-TESE tissue specimens were received, all the above described steps were repeated. A minimum of two laboratory technicians/embryologists at a time handled the micro-TESE specimens: one used to mince the tubules under stereomicroscopy and the other searched for spermatozoa under the inverted microscope. If no spermatozoa were observed after initial microscopic examination, extensive mechanical processing and searching were undertaken. Cell suspensions were diluted with sperm medium and centrifuged at x300 g for 7 minutes. The obtained supernatants were discarded 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 searching of sperm, and each microdroplet was loaded with approximately 1 μ L of testicular cell suspension. The temperature of culture media during sperm handling and processing were kept in the range of 32-37°C. When spermatozoa were identified, they were picked up for ICSI using the microinjection micropipette and transferred to a microdroplet of polyvinyl pyrrolidone. A group of pre-selected spermatozoa for ICSI was taken and morphologic sperm assessment was performed in them. Selected spermatozoa were then immobilized, aspirated into the micropipette and inject into the cytoplasm of metaphase-II oocytes. The sperm immobilization was carried out by firmly touching the tip of injection pipette to the transition zone between mid-piece and sperm tail [22]. These injected oocytes were transferred to a closed culture system and incubated for 16-18 hours at 37°C and 5.5% CO₂, until confirmation of fertilization.

Fertilization was considered to be normal when oocytes with two pronuclei were seen. Embryo cleavage was checked at approximately 48 and 72 hours after ICSI and the number, symmetry, and expansion of the blastomeres, multinucleation, anomalies of the zonapellucida, and the rate of cytoplasmic fragmentation were recorded. The embryos were classified as top quality when three to four symmetrical blastomeres on the second day of culture and seven to eight symmetrical blastomeres on the third day were seen, with no multinucleation, grade I (no fragmentation) or grade II fragmentation (up to 20% of the perivitelline space with fragments), and no abnormalities in the zona pellucida [23].

Ovarian Stimulation, Oocyte Retrieval and Embryo Transfer:

For all cycles, GnRH antagonist protocol was used. All patients were pretreated with oral contraceptive pills containing ethinyl estradiol 30 mcg and levonorgestrel 150 mcg for 15-21 days. For ovarian stimulation, initial daily doses of 150-375 IU of recombinant human FSH (Gonal-F[®], Merck Serono, India) were utilized. The initial dose of gonadotropin was determined by the treating physician taking into account various 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 pre-antral follicles seen on Transvaginal Ultrasound Scan (TVUS) on days 2 or 3 of the menstrual cycle prior to ovarian stimulation. Ultrasound assessment between the fifth and eighth days of stimulation was performed to determine if gonadotropin dose adjustments were needed; if prevention of ovarian hyper-response was deemed necessary, the dose was reduced. There was, however, no dose increase of gonadotropin during stimulation, even in cases of poor ovarian response. Once the lead follicle reached 14 mm, GnRH antagonist 0.25 mg (Cetrootide[®], Merck Serono, India) was added and continued till the day of final injection. Human chorionic gonadotropin was administered when two or more ovarian follicles reached a mean diameter of 17 mm or more. Recombinant chorionic gonadotropin 250 μ g (Ovitrelle[®], lyophilized, Merck Serono, India) was used for the final maturation of oocytes. Oocyte retrieval was performed under local anesthesia along with IV sedation and guided by TVUS, 35 hours after hCG administration. After oocyte aspiration, the protocol for ICSI, embryo transfer and cryopreservation was same as previously described elsewhere [15].

Definitions and criteria

Success on micro-TESE was reported as the acquisition of any number of motile or immotile spermatozoa that allowed sperm injections to be performed. A minimum of 20 seminiferous tubules cross-sections were evaluated on histopathology. Both the predominant and the most advanced stage of spermatogenesis were noted. Sertoli cell-only (SCO) category denoted that tubules were lined with Sertoli cells and devoid of germ cells. Maturation arrest (MA) was defined as absence of mature spermatozoa, despite having normal early stages of spermatogenesis. Normal spermatogenesis was taken 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 6 to 7 week. Miscarriage was considered when nonviable clinical pregnancy was noted on ultrasound before gestational week 20.

Data collection

Data collection was done prospectively. Demographics and baseline characteristics of patient population, success of sperm acquisition,

presence of motile sperm and surplus sperm for cryopreservation were noted. The following outcomes were also noted 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 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 their confidentiality guaranteed. The study was exempted from IRB approval since it involved the analysis of records from already established clinical practices.

Statistics

Patient demographics and outcomes of sperm injection cycles were analyzed descriptively. Mann-Whitney U test and Chi-Square test were utilized to compare demographic parameters and baseline characteristics of men with successful and failed sperm retrievals. A $p < 0.05$ was considered significant. All statistical data were processed with SPSS 16.0

Results

Micro-TESE was successful at obtaining testicular sperm in 54.4% (98/180) of cases. Among patients with previous failed retrievals by TESA/ open TESE methods, micro-TESE was successful in 56.7% (67/118). Sperm retrieval was successful in nearly 50% of cases in the etiological categories of post-orchidopexy (12/20), post-orchitis (17/32) and idiopathic (46/95). Presence of motile spermatozain cases with successful sperm retrieval was 69.3% (68/98). Cryopreservation of surplus retrieved sperm was carried out in 63(54.3%) cases. Patients' demographic characteristics, baseline endocrinal profile, testicular volume, and the operative characteristics of patients with successful and failed micro- TESE are presented in Table 1.

In 84 (46.6%) of the cases, micro-TESE was performed on both

testes. Mean operative duration was 78 minutes (range 39-124) and 144 minutes (range 114-192) minutes for unilateral and bilateral cases, respectively. Of these, sperm retrieval was successful within the first 2 hours of operation in 72/98 (73.4%) cases. The mean number of testicular fragments excised was 11 (range 4-22). The MA cases exhibited uniform seminiferous tubule size and opacity. Hence, excised tissue fragments were taken at random since no clear distinction among tubules was possible using the operating microscope, and in 40% of them testicular sperms was retrieved. Of the 63 cases of SCO, a clear distinction between collapsed and normal appearance tubules was possible in 19 cases. Of them, testicular sperm were retrieved in 14 (73.6%) cases. In all but 4 cases, spermatozoa were identified at initial laboratory screening. In these 4 cases, sperm was found after extended laboratory processing after a failed initial screening. No major complications were noticed after micro-TESE procedures. Amongst the minor complications, pain was the commonest complaint and all patients had mild scrotal edema.

Of the 79 men who received medical therapy before micro-TESE, 48/79 (60.7%) had initial testosterone levels < 300 ng/dL and 31/79(39.2%) had T/E ratio < 10 . Among the treated individuals, 61/79 (77.2%) responded and had either serum testosterone levels > 300 ng/dL or T/E ratios > 10 before micro-TESE procedure, while 18/79 (22.7 %) did not. Sperm Retrieval Rates (SRR) were higher in the group who responded compared to the one who did not respond to medical therapy, 80.3% (49/61) vs. 38.8% (7/18); $p = 0.0021$ two-tailed Fisher exact). Similarly, SRR were higher in patients who received medication to boost testosterone production compared with those who did not, 53.1% (42/79) vs. 35.6% (36/101); $p = 0.02$, two-tailed Fisher exact). Preoperative levels of FSH were significantly lower in men with successful retrieval compared to ones with failed retrievals (16.3 ± 16 vs. 21.0 ± 15.9 mIU/ml, $p = 0.018$). There were no differences between patients with success or failure of sperm retrieval in micro-TESE with respect to the presence of varicocele, and male baseline characteristics. Retrieval rates differed according to the results of testis biopsy taken during the operations. Patients with Sertoli cell-only (41%) and Maturation arrest (40%) had lower SRR compared with hypospermatogenesis (100%). Comparative rates are presented in Table 2.

	Successful micro-TESE (n=98)	Failed micro- TESE (n=82)	P value
Male Age (years)	37.4 ± 5.7 (24;52)	36.3 ± 5 (28;49)	0.247
Testicular Volume (cc)	10.5 ± 3.7 (2.5;20)	9.6 ± 4 (1.5;16)	0.214
Baseline Hormone			
FSH(mIU/mL)	16.3 ± 16 (0.29; 101.37)	21.0 ± 15.9 (0.42;67.96)	0.018*
LH(mIU/mL)	5.6 ± 4.1 (0.10; 24.08)	7.0 ± 4.6 (0.10;18.14)	0.053
Estradiol(pg/ml)	37.2 ± 27.7(5.01;210.0)	36.6 ± 19.3(5.0;88.65)	0.475
Total testosterone (ng/dL)	460.1 ± 220.6 (86; 1198.0)	417.8 ± 199.5 (94;1100)	0.297
T/E ratio	23.1 ± 21.6	25.8 ± 29.52	0.624
No. Bilateral retrieval (%)	14 (14.3)	78 (95.1)	0.0001*
Seminiferous tubules diameter (microns)	241.2 ± 77.4(119.0;498.0)	188.3 ± 73.5 (87.0;380.0)	$< 0.001^*$

*Statistical significant difference between the groups. Values are mean ± SD

Table 1: Demographics and operative characteristics of patients with successful and failed micro- TESE

	Successful micro -TESE (n=98)	Failed micro-TESE (n=82)
Sertoli – cell only (%)	26 (41.3)	37 (58.7)
Maturation arrest (%)	30 (40.0)	45 (60.0)
Hypospermatogenesis (%)	42(100)	0(0)

Table 2: Sperm retrieval rate in relation with testicular histology.

Bilateral micro-TESE procedures was required in only 14.4% (14/98) of the successful retrieval group compared with 95% (78/82) of the failed group ($p=0.0001$). Four patients in the failed group did not have bilateral micro-TESE due to solitary testis. The average maximum seminiferous tubule diameter was higher in the group with successful (241.2 ± 77.4 microns) compared with failed retrievals (188.3 ± 73.5 microns; $p<0.001$)

Sperm injections were carried out for 94 couples using testicular sperm retrieved by micro-TESE. The remaining 4 cases had severely abnormal sperms not fit for ICSI. The baseline characteristics of female partners of successful micro-TESE cases are presented in Table 3. Injections were performed using motile sperm in 72.3% (68/94) cases. In the remaining cases, injections were carried out using the hyposmotic swelling test to assess sperm vitality [9,21]. Normal two pronuclei fertilization rate after sperm injections was 51%. The cleavage rate of fertilized zygotes was 75%. Fresh transfers were performed for 72 cases with an average of 2.0 ± 0.72 embryos replaced to the uterine cavity on day 4 or 5 depending on embryo quality. Embryos were cryopreserved if the patient was at high risk of OHSS or if the endometrium was less than 7 mm or if serum progesterone was higher than 1.5 ng/ml on the day of final maturation trigger. These patients underwent frozen thaw embryo transfer in subsequent cycles with an average of 2.3 ± 0.45 embryos. Cumulative clinical pregnancy rate per ICSI cycle with sperm injection and embryo transfer was 29.78% (28/94), with an implantation rate of 19% considering fresh and frozen-thawed transfers. Among the cases with failed sperm retrieval, the retrieved oocytes were either vitrified or discarded depending on the couple's preference. The sperm injection outcome of female partners of successful micro-TESE cases are presented in Table 4.

	Successful micro-TESE (n=98)
Female age (years)	30.10 ± 5.4
Infertility duration (years)	6.9 ± 3.0
Baseline hormone	
FSH (mIU/mL)	6.6 ± 2.2
LH(mIU/mL)	2.8 ± 2.3
AMH(ng/mL)	2.7 ± 2.6

Table 3: Baseline characteristics of female partners of successful micro-TESE cases.

	Successful micro-TESE (n=98)
No. ICSI cycles	94
No. \pm SD Oocytes retrieved	12.4 ± 5.5
No. \pm SD Injected oocytes	10.2 ± 4.8
No. \pm SD 2PN fertilized oocytes (%)	6.1 ± 3.5 (61)
No. Cleaved pre-embryos (%)	435 (75)
No. \pm SD Transferredembryos (Fresh)	2.0 ± 0.72
Transferred Embryos(Frozen)	2.3 ± 0.45
Cumulative clinical pregnancy rate/ICSI cycle* (%)	28 (29.78%)
Implantation rate (%)	19.0
Ongoing PR > 20 weeks (%)	26 (26.3)

* consisting of all fresh and frozen embryo transfers

Table 4: Sperm injection outcome of female partners of successful micro – TESE cases.

Discussion

Non-obstructive Azoospermia (NOA) is one of the most difficult conditions to deal with pertaining to male infertility. It leads to disruption of spermatogenesis. Sperm Retrieval (SR) coupled with intracytoplasmic sperm injection (ICSI) is the only valid option for such patients seeking self-parentage. Microdissection Testicular Sperm Extraction (micro-TESE) technique has been shown to be more efficient than all other modalities of SR in NOA [24].

Micro-TESE was found to be a reliable method to obtain sperm for ICSI in our group of men with NOA. A cumulative SRR of 54.4% was obtained and sperm injections using the retrieved testicular sperm were carried out in all but four cases. Normal fertilization after sperm injections was achieved in 51% of the oocytes, and morphologically normal cleaved embryos for transfer were available for all but three couples. Among the 94 ICSI cycles with successful sperm retrieval and embryo transfers, a cumulative ongoing pregnancy rate of 29.78% was obtained in this series.

Our study depended primarily on the micro –TESE method applied to men with NOA and poor prognosis for SR. Non-obstructive azoospermia is an untreatable condition associated with testicular failure. It comprises a spectrum of testicular histopathology resulting from various causes including, but not limited to, genetic and congenital abnormalities, post-infectious, exposure to gonadotoxins, trauma, endocrine disorders, and idiopathic [16]. The only available option for men with NOA to achieve biological fatherhood is surgical sperm retrieval and ICSI. The population of azoospermic men seen in our daily practice consists mainly of couples who do not accept gamete donation as our center practices a strict non-donor policy. Therefore, we opted to implement the microsurgical method for sperm retrieval due to the reported higher effectiveness and lesser adverse effects of this procedure to retrieve testicular sperm in NOA. Literature quotes retrieval rates ranging from 35% to 77% for micro-TESE, and more importantly, controlled series have consistently demonstrated that micro-TESE performed better than conventional sperm extraction (TESE) or percutaneous aspirations (TESA) [2,3,6,10-14,18,25]. In our initial series of patients, we achieved 50% successful sperm retrieval with an acceptable cumulative clinical pregnancy rate per ICSI cycle of 28.6% [15]. The positive start encouraged us to go further in helping the men with NOA achieve fatherhood. 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 [2,3,26]. In this study, we confirmed the diagnosis of NOA by histology, and most of the included patients had the worst cases scenarios of failed prior retrievals and unfavourable testicular histology. Comparable success rates have been achieved among different etiology categories of cryptorchidism, varicocele, orchitis, genetic, radio-/chemotherapy and idiopathic [19,27-29]. Furthermore, micro-TESE has been shown to be successful in approximately 1/3rd of previous failed retrievals by other methods [9,10]. Here, we were successful in 50% of our retrieval attempts in the etiology categories of post-orchidopexy (cryptorchidism), post-orchitis and idiopathic, and in 2/3 of cases with previous failed retrievals by other methods. The most important parameter determining successful sperm retrieval seemed to be the age at which orchidopexy was done as it has been noted that the mean age at orchidopexy significantly differed in men with positive (10.6 years) and negative (15.5 years) sperm recovery [30].

Furthermore, surplus sperm for freezing were available in more than half of the men with successful retrievals. While cryopreservation

	Micro TESE	Open TESE
Set up	Dedicated OT	Not a must
Operating microscope	Needed	Not needed
Operating expertise	Dedicated surgeon needed	Not a necessity
Operating time	1-4 hours	15-60 minutes
Tissue removed	less	more
Testicular damage	less	more
Successful retrievals	Nearly 50%	30%

Table 5: Micro TESE vs. Open TESE.

may prevent the need for future retrievals in case ICSI fails, some authors argue that fresh testicular sperm is preferable for ICSI because frozen-thawed surgically-retrieved sperm from NOA men have lower reproductive potential compared with fresh counterparts [31,32]. In a recent study, however, comparing sperm injections with fresh and frozen micro-TESE spermatozoa no difference was seen in fertilization rate and pregnancy rates [33,34].

If needed, repeat micro-TESE is a valid option. In such cases it is advisable to allow an interval of at least 6 months between retrievals. In a study that compared retrieval rates in repeat procedures, micro-TESEs were more likely to be successful after 6 months compared with those performed within 6 months (8% vs. 25%) [8].

The options for sperm retrieval in difficult cases of NOA are either to remove larger amounts of tissue or to microsurgically identify morphologically normal seminiferous tubules that are more likely to harbor sperm production [2,10]. While we were successful overall in our retrieval attempts with only minor postoperative complications, micro-TESE has certain advantages as well as disadvantages when compared to other open surgical methods (Table 5). Micro-TESE is a labor-intensive procedure that requires microsurgical expertise and an operating room equipped with a top-quality operating microscope. In our series, the mean operative time was approximately 2 hours; however, operations ranged from 39 minutes to more than 3 hours. We were successful in retrieving sperm within the first 2 hours in 73.4% of cases. Furthermore, spermatozoa was identified at initial laboratory screening in all but four cases that required extended laboratory processing to find sperm after a failed initial screening. These results are in similar lines to recent series, including our own preliminary experience, in which the best chance of sperm recovery during micro TESE occurred within the first 2 hours of the operation [35]. The length of time needed for the procedure might be the reason deterring the widespread applicability of micro-TESE [19]. On the other hand, the use of operating microscope during micro-TESE reduces the chances of vascular injury by proper identification of testicular vessels under the tunica albuginea before the incision is placed. Excellent hemostasis achieved through microsurgery reduces the chances of hematoma formation and testicular devascularization as it may occur in cases of conventional TESE [2,12,26]. Additionally, the amount of removed testicular parenchyma in micro-TESE is significantly lessened in micro-TESE compared with conventional TESE. It had been shown that micro-TESE compared to open TESE results in 3 times higher number of sperms retrieved with 70 times lesser testicular tissue removed [35]. Excessive tissue removal may adversely affect androgen production and jeopardize the chances of repeated sperm retrieval which are particularly important for men with NOA who usually have small and highly dysfunctional testes [8]. From the laboratory perspective, the small amount of tissue extracted by micro-TESE facilitates sperm processing and search. For TESE, sample processing may be incredibly labor-intensive and the searching process

may miss the rare spermatozoa within a sea of seminiferous tubules and plethora of other cells [19,35]. It has been reported that the optimal fertilization by ICSI using surgically-retrieved sperm is achieved when the time frame from hCG trigger administration to microinjection does not exceed 44hours [36]. For this reason, operations and laboratory processing of extracted specimens should be coupled with oocyte aspiration and sperm injections in a coordinated manner to avoid losing the precious time. It is far easier to select spermatozoa from a smaller population of contaminating testicular cells, thus, facilitating the laboratory steps of this complex process [21]. Similarly, in this present series, we found it to be 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 for a prolonged duration. A clear-cut advantage of micro-TESE is the ease for sperm search, less contamination and blockage of the microinjection needle with unwanted cells and debris that resulted in greater speed for sperm pick-up and injections.

To date, the search for an ideal preoperative predictive factor for successful SR in NOA is far from over. The often used trio of follicle-stimulating hormone, testosterone levels and testes volume is not suitable because they reflect a global testicular function and does not exclude the presence of a site of normal sperm production within a dysfunctional testis [37,38]. Testicular histopathology results, on the other hand, confer better prognostic value compared with the aforesaid markers, and serve as one of the most direct markers of spermatogenesis or lack of it. Sperm retrieval rates by micro-TESE varies according to the histopathological alteration, and are significantly higher in hypospermatogenesis (93%) compared to maturation arrest (64%) and Sertoli-cell only syndrome (20%) [25]. An adverse histopathology should not be a deterrent to offer SR, as successful retrievals are reported even in the more adverse histopathology pattern of SCO, as shown in the aforementioned study and also in our own preliminary results [15].

This could be due to the fact that sperm production is thought to be distributed in a heterogeneous pattern within the testis. Therefore, histologic assessment of a single testicular fragment is unlike to be representative of other areas, and will be limited in its ability to determine the presence of rare foci of sperm production in NOA [2,25]. We were able to retrieve testicular sperm in more than 1/3 of the men in which the predominant histopathology pattern was SCO or maturation arrest. Interestingly, sparse seminiferous tubules containing foci of germ cells were observed in SCO cases, thus reaffirming the heterogeneity of sperm production within the testis. In our series, the diameter of the excised seminiferous tubules was the standalone parameter associated with the chance of obtaining sperm. Notably, a clear distinction between collapsed and normal appearance/dilated tubules was possible in 1/3 of our cases and was associated with excellent sperm retrieval rate. Conversely, the presence of a uniform pattern of collapsed tubules with none different in size was associated with failed retrievals in majority of cases. Histologic examination revealed that such cases were those of Sertoli cell-only. Along the same lines, we were unable 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 those with maturation arrest in which seminiferous tubules contained germ cell and were therefore normal in size. Hence, it was impossible to distinguish the ones which have full maturation to spermatozoa, if any, by using the operating microscope [2]. Our results showing greater tubule diameter in cases of successful retrievals are consistent with a recent report in which the mean maximal diameter

of sperm-containing seminiferous tubules was significantly higher than non-sperm containing tubules (298 vs. 225 microns, $P < 0.0001$) [39]. In the same study, a cut-off level of 250 microns was found to have the best sensitivity and specificity for positive sperm retrieval [39]. Our findings highlight the usefulness and limitations of the operating microscope to identify areas containing larger tubules in size within the testis which are having higher probability to harbor sperm production. In addition, the histopathology data can be used as a counseling tool for doctors dealing with men with NOA seeking fertility advice as for the presence of Sertoli cell-only is not an indicator of absolute sterility and possibility of successful SR exists.

Alteration of intratesticular hormonal milieu to enhance testosterone production by medical therapy prior to sperm retrieval has been suggested to increase chances of successful retrieval in men with NOA [1,34]. The rationale of same relies on the fact that most men with NOA have lower volume testes which is associated with hampered testosterone production and hypogonadism. Adequate levels of intratesticular androgenic bioactivity are thought to be essential to sustain spermatogenesis that might be compromised in NOA [40]. Drugs utilized for the same include aromatase inhibitors, clomiphene citrate and human chorionic gonadotropin to boost testosterone production in men with NOA and non-mosaic Klinefelter Syndrome (KS). SR rates have been reported to be increased by 1.4-fold in KS men who responded to medical therapy compared to ones who did not [34]. Nearly 44% of men in our series had signs of hypogonadism, as indicated by either low serum testosterone or abnormal testosterone to estradiol ratios, and received medical therapy prior to micro-TESE. Medical treatment prior to micro-TESE has been utilized widely in men with NOA [40]. Our group of men with hypogonadism who received medication to boost testosterone production had higher SRR compared with those without hypogonadism and no medical therapy. Moreover, we found that a positive response to medical therapy, characterized by a significant increase in total testosterone levels from baseline, was associated with higher SRR response to medical therapy. Our findings are in accordance with a recent study involving 442 men with NOA who received medication prior to SR. The authors of the aforementioned study aimed at achieving 600-800 ng/dl testosterone levels post treatment, and reported SRR of 57% vs. 33.6% in the treated and untreated groups [41].

Despite our positive results, the role of medical treatment prior to micro-TESE is still investigational, and contrary results have also been reported. Of note, in a large retrospective study on the role of optimizing testosterone before micro-TESE in men with NOA, Reifsnnyder and co-authors evaluated 736 individuals and concluded that hormonal therapy had no impact on retrieval rate [1,40].

At the moment, a definitive conclusion cannot yet be drawn on the role of medical therapy in NOA until adequately powered randomized trials including different subsets of men with NOA in whom intratesticular androgenic activity is measured solve this dilemma.

After retrieval of testicular sperm from men with NOA, ICSI is preferred over IVF due to paucity of available sperms for injection. In our series, fertilization and embryo development after ICSI were in agreement with those reported in the literature and an ongoing cumulative pregnancy rate per transfer of 26.5% has been reassuring [1,3,9,27-29]. The reported fertilization and implantation rates are lower in NOA compared with obstructive azoospermia and ejaculated sperm due supposedly to associated severe morphological defects [3,9].

Same is true for clinical pregnancy and live birth rates as well compared to aforementioned male infertility scenarios [3,9,42]. The likelihood of achieving a live birth was found to be 1.8-fold higher (Odds Ratio [OR]=1.86; 95% Confidence Interval [CI]: 1.03-2.89) in men with OA compared to men with NOA [42]. These findings indicate that men with NOA undergoing ART have significantly decreased reproductive potential. This may be due to a higher tendency of sperm carrying deficiencies related to the centrioles and genetic material. Subsequently, activation of the egg and trigger formation and development of a normal zygote and a viable embryo with these sperms might be impaired [43]. Nevertheless, the neonatal outcome of babies born does not seem to be affected by the type of azoospermia or the source of sperm used for ICSI. Malformation and perinatal death rates in NOA are in the range of 1.3%-5.2% in large cohorts [44,45]. Given the fact that the currently available data is based on a very limited population of children born, continuing monitoring in larger groups is warranted.

Our results with micro-TESE in a large group of men are reassuring and the information provided can be useful for doctors treating male infertility who wish to improve the chances of sperm retrieval during TESE. 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 sperm retrieval are to obtain the adequate number of good quality sperm for immediate use and/or potential cryopreservation while minimizing the damage to the reproductive tract. In non-obstructive azoospermia, sperm production can either be markedly impaired or absent. As such, open surgical testicular sperm retrieval with or without microscopic magnification is recommended to optimize the chances of finding sperm. Our data reaffirm the existing knowledge that micro-TESE provides success in more than 50% of men with NOA. In our series, micro-TESE was associated with only few minor complications, and with marked reduction in time processing of testicular specimens. Micro-TESE has shown to be an outstanding method to retrieve spermatozoa from the most severe cases of NOA.

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Authors' contributions

MA, DR and SS were responsible for the manuscript's concept, design, supervision and review of intellectual content. SC, SR, VD and DR were responsible for literature search, data acquisition and critical review of content. SR performed all the embryology related work. SC, S and SS performed data analysis and statistics, and also prepared and edited the manuscript. All authors reviewed and approved its final version. SC, DR, and SS take responsibility for the integrity of this work and should be designated as "guarantors".

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