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E-mail: jzus@zju.edu.cn

Correspondence:

Modified stepwise mini-incision microdissection testicular sperm extraction: a useful technique for patients with a history of orchidopexy affected by non-obstructive azoospermia*

Peng LI[§1,2], Chen-cheng YAO[§1], Er-lei ZHI[1], Yuan XU[3], Zhong WAN[1], Ying-chuan JIANG[1,4], Yu-hua HUANG[1], Yue-hua GONG[1], Hui-xing CHEN[1], Ru-hui TIAN[1], Chao YANG[1], Liang-yu ZHAO[1], Zheng LI[†‡1,3]

[1]*Department of Andrology, Center for Men's Health, Urologic Medical Center, Shanghai General Hospital, Shanghai Jiao Tong University, Shanghai 200080, China*
[2]*Department of Urology, Shanghai General Hospital of Nanjing Medical University, Shanghai 200080, China*
[3]*Reproductive Medicine Center, Shanghai General Hospital, Shanghai Jiao Tong University, Shanghai 200080, China*
[4]*Department of Urology, Zhoushan Hospital, Zhoushan 316021, China*
[†]E-mail: lizhengboshi@sjtu.edu.cn

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Non-obstructive azoospermia (NOA), which is defined as the absence of spermatozoa in the ejaculate secondary to impaired spermatogenesis within the testis, may be caused by a variety of etiologies, including varicocele-induced testicular damage, cryptorchidism, prior testicular torsion, post-pubertal mumps orchitis, gonadotoxic effects from medications, genetic abnormalities, chemotherapy/radiation, and other unknown causes currently classified as idiopathic (Cocuzza et al., 2013). The microdissection testicular sperm extraction (micro-TESE) technique involves a meticulous microsurgical exploration of the testicular parenchyma to identify and selectively extract larger seminiferous tubules that carry a higher probability of complete spermatogenesis (Schlegel, 1999). The Cornell group evaluated the efficacy of micro-TESE in 152 NOA patients with an associated history of cryptorchidism. In their series, spermatozoa were successfully retrieved in 116/181 attempts (64%), and the resulting pregnancy rate was 50% with a delivery rate of 38% (Dabaja and Schlegel, 2013). Franco et al. (2016) described a stepwise micro-TESE approach in NOA patients, which was considered to reduce the cost, time, and effort associated with the surgery. Alrabeeah et al. (2016) further reported that a mini-incision micro-TESE, carried through a 1-cm equatorial testicular incision, can be useful for micro-TESE candidates, particularly in patients with cryptozoospermia. We conducted a retrospective study of 20 consecutive NOA patients with a history of orchidopexy from May 2015 to March 2017. We employed a modified stepwise mini-incision micro-TESE consisting of three steps: (1) mini-incision micro-TESE; (2) standard micro-TESE of the ipsilateral testis; (3) contralateral micro-TESE. The operating time was evaluated, and it revealed statistically significant differences between the mini-incision micro-TESE group ((52±23) min), the standard micro-TESE group ((87±10) min) and the bilateral-standard micro-TESE group ((121±9) min) (mini-incision micro-TESE group vs. standard micro-TESE group, $P<0.05$; mini-incision micro-TESE group vs. bilateral-standard micro-TESE group, $P<0.001$). The overall sperm retrieval rate (SRR) was 80% (16/20). In 50% of patients (10/20) mini-incision

‡ Corresponding author
§ The two authors contributed equally to this work
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ORCID: Peng LI, https://orcid.org/0000-0001-7640-8254; Chen-cheng YAO, https://orcid.org/0000-0002-8820-7895; Zheng LI, https://orcid.org/0000-0001-6735-1831

micro-TESE alone was required because of the rapid identification of spermatozoa harvested from the superficial testicular tissue. The remaining 10 patients underwent a standard micro-TESE, and 4 patients progressed to a bilateral micro-TESE. The sperm were cryopreserved for future intracytoplasmic sperm injection (ICSI), and, to date, 10 of 16 couples have attempted ICSI. Clinical pregnancies were achieved in 5 of the 10 couples, whereas ongoing pregnancy or deliveries occurred in 4 of the 10. Mini-incision micro-TESE can prove sufficient, reserving standard micro-TESE as a salvage method, for patients affected by NOA with a history of orchidopexy. The modified stepwise mini-incision micro-TESE, which gradually increases surgical invasiveness, may represent an optimal approach for sperm retrieval for patients affected by NOA with a history of orchidopexy.

Cryptorchidism is the most common genital anomaly in boys. The diagnosis is rendered when one or both testes fail to descend to a normal scrotal position during development (Wood and Elder, 2009; Serrano et al., 2013). The incidence of NOA in men with unilateral cryptorchidism is 13% regardless of whether the patient was treated or not; in the most severe cases, approximately 88% of patients with untreated bilateral cryptorchidism will develop into azoospermia (Hadziselimovic and Herzog, 2001). Fortunately, surgical orchidopexy is associated with a success rate exceeding 80% (Docimo, 1995). Despite best efforts, 10% of infertile men still have a history of cryptorchidism and orchidopexy (Grasso et al., 1991), probably due to the loss of Ad spermatogonia (Hadziselimovic and Herzog, 2001).

All patient characteristics are listed in Table 1. No genetic abnormalities were present. Average age (mean±standard deviation (SD)) was (30.7±5.5) years (range from 23 to 47 years). The average age at orchidopexy (mean±SD) was (12.8±9.3) years (range from 3 to 35 years). In the 20 men, 13 suffered from bilateral cryptorchidism whereas the remaining 7 had unilateral cryptorchidism. The average right testicular volume (mean±SD) was (7.1±2.1) mL, and the left was (7.6±2.3) mL. Notably, one patient had a history of radiotherapy/chemotherapy for left testicular carcinoma. Among the study subjects, 10 required mini-incision micro-TESE alone, 6 underwent mini-incision and ipsilateral standard micro-TESE, and the remainder had standard bilateral micro-TESE procedures. All surgical procedures were carried out under general anesthesia. Generally, the larger testicle, determined by physical examination and/or prior scrotal ultrasound, was explored first. A longitudinal 3-cm scrotal incision was made over the right or left testicle (a contralateral incision was made if a bilateral procedure proved necessary) (Fig. 1a), not the median raphe incision (Fig. 1b, the dotted line). The subcutaneous tissue was then gently dissected to expose the testis, which was fixed outside of the dartos muscle given the prior history of orchidopexy. The testicle was delivered, which at times was difficult due to scarring. A planned transverse, equatorial, approximately 1-cm incision ("mini-incision") was then carried down through the tunica albuginea to expose the testicular parenchyma under the operating microscope (Fig. 1d). The available testicular tissue beneath the mini-incision was examined under the operating microscope at 15× to 24× magnification to locate and collect dilated seminiferous tubules (Fig. 1e). A specimen was only collected if dilated tubules were identified, which was immediately evaluated by an embryologist available in the operating room. If spermatozoa were present, the motility, morphology, and quantification were provided. Usually, the surgeon would examine the slides with the embryologist to confirm the result. If no spermatozoa were found in this superficial exposure, the incision was then extended to perform the standard micro-TESE (Fig. 1f). Failure resulted in a full contralateral micro-TESE. When sperm was found, it was cryopreserved for future ICSI. The procedure was terminated when several spermatozoa were observed or after complete and thorough examination of the entire testicular parenchyma. At the completion of the procedure, the tunica albuginea and skin were closed with 5–0 suture (Ethicon, Puerto Rico, USA). If sperm were present, they were cryopreserved using either the LSL straw (Cryo BioSystem, Paris, France) (Liu et al., 2017) or the cryopiece system (Sun et al., 2017), according to the quantity and quality of the spermatozoa available for ICSI.

The operating time among the mini-incision micro-TESE group ((52±23) min), the standard micro-TESE group ((87±10) min) (mini-incision micro-TESE group vs. standard micro-TESE group, $P<0.05$) and the bilateral-standard micro-TESE group ((121±9) min) (mini-incision micro-TESE group vs.

Table 1 Clinical characteristics of the 20 NOA patients with a history of orchidopexy

No.	Age (year)	Age at orchidopexy (year)	Cryptorchidism	FSH (IU/L)	LH (IU/mL)	T (ng/mL)	Surgical procedure	Operation time (min)	Sperm	ICSI	Clinical pregnancy	Live birth
1	35	10	Unilateral	15.05	5.77	3.27	Mini	65	Positive	Y	P[d]	
2	26	19	Unilateral	20.12	11.79	2.86	Mini	30	Positive	Y	N	
3	30	3	Unilateral	6.60	10.18	11.46	Mini	45	Positive	Y	N	
4	29	6	Unilateral	1.78	2.62	7.61	Mini	50	Positive	Y	P	Abortion
5	31	6	Bilateral	11.85	8.32	2.02	Mini	80	Positive	Y	N	
6	35	6	Bilateral	5.68	5.97	8.07	Mini	105	Positive			
7	47		Unilateral	8.17	1.30	3.83	Mini	40	Positive			
8	31	11	Bilateral	33.56	7.20	3.94	Mini	45	Positive	Y	P	Y (girl)
9	26	23	Bilateral	6.43	3.62	6.71	Mini	30	Positive			
10	30	5	Unilateral	1.34	3.00	15.63	Mini	30	Positive	Y	P	Y (girl)
11	23	22	Bilateral	7.98	3.62	5.06	Bi-Stan	120	Positive			
12	25	10	Bilateral	28.94	6.73	2.82	Stan	80	Positive	Y[c]		
13	34	6	Bilateral	25.73	10.85	2.41	Stan	90	Positive	Y[c]		
14[a]	31	8	Bilateral	26.20	12.21	4.32	Stan	100	Positive	Y	P	Y (boy)
15	28		Bilateral	84.59	4.39	4.39	Stan	80	Positive	Y	N	
16	39	35	Bilateral	51.10	34.06	7.73	Stan	75	Positive	Y	N	
17	27	5	Bilateral	22.63	7.11	1.96	Bi-Stan	110	Negative			
18	27	15	Bilateral	9.12	4.87	2.33	Bi-Stan	135	Negative			
19	35	32	Bilateral	68.60	37.54	1.12	Bi-Stan	120	Negative			
20	25	8	Unilateral	24.53	61.92	5.92	Stan	100	Negative			
Average[b]	30.7± 5.5	12.8± 9.3		19.4± 18.9	12.2± 14.7	5.1± 3.3		76.5± 32.5				

NOA: non-obstructive azoospermia; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone; ICSI: intracytoplasmic sperm injection; Mini: mini-incision micro-TESE; Stan: standard micro-TESE; Bi-Stan: bilateral standard micro-TESE; Y: yes; N: no; P: pregnancy. [a] History of radiotherapy/chemotherapy for a left testicular carcinoma; [b] Data are expressed as mean±standard deviation; [c] Oocyte frozen, immotile sperm post-thawing; [d] Ongoing pregnancy

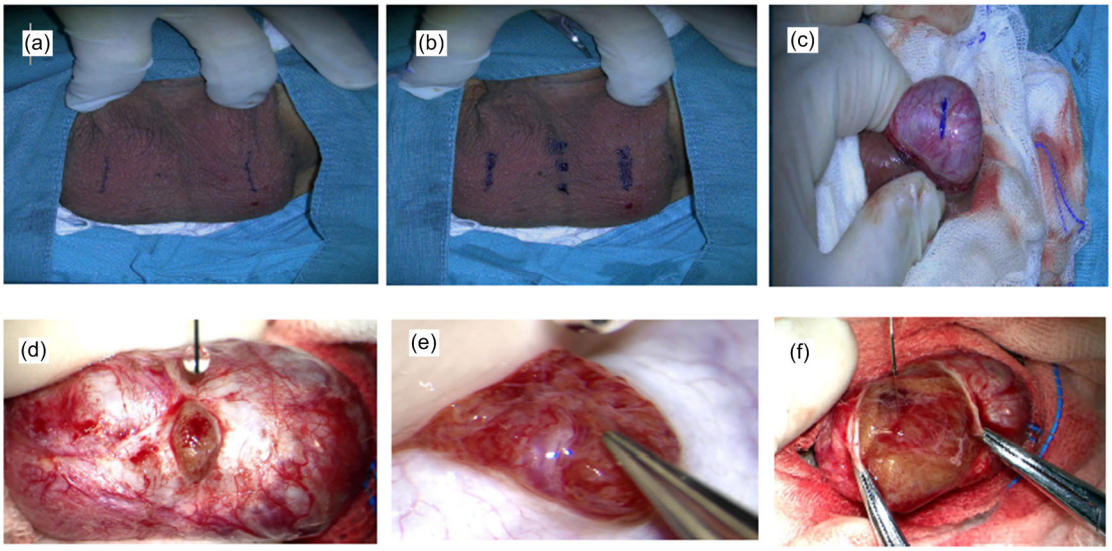

Fig. 1 Photographic image demonstrating the modified stepwise mini-incision micro-TESE

(a, b) Photograph showing the pair of longitudinal scrotal incisions made just over the right or left testicle (solid line), and the median raphe incision (dotted line); (c, d) Photograph showing the planned equatorial testicular mini-incision; (e) Photograph of the mini-incision micro-TESE; (f) Photograph showing the mini-incision extended to the standard micro-TESE incision

bilateral-standard micro-TESE group, $P<0.001$) was evaluated (Fig. 2), and it indicated that the stepwise mini-incision micro-TESE could reduce operating times markedly. SRR has been documented to be between 51.9% and 73.0% (Lin et al., 2001; Negri et al., 2003; Vernaeve et al., 2004; Haimov-Kochman et al., 2010). Raman and Schlegel (2003) reported that spermatozoa were successfully retrieved in 35 of 47 TESE attempts (74%), including 5 of 8 (63%) attempts made by the multiple testicular biopsy technique, and 30 of 39 (77%) attempts by the micro-TESE method. The Cornell group reported an SRR of 64% (116/181) in 152 patients with NOA associated with cryptorchidism. Their resulting pregnancy and delivery rates were 50% and 38%, respectively (Dabaja and Schlegel, 2013). Findings suggest that NOA patients, after correction of maldescended testes, tend to have a significantly higher SRR (80%) than men with a primary fine needle biopsy (FNB) diagnosis of spermatogenic arrest (SRR 40%–45%) and a clearly higher SSR than men with Sertoli cell only (SRR 30%–35%) (Bernie et al., 2013). Due to rapid identification of spermatozoa harvested from the superficial testicular tissue, 50% (10/20) required only a mini-incision micro-TESE. However, of men who required the full bilateral micro-TESE approach, spermatozoa were successfully retrieved in only one of four. The SRR was 85.7% (6/7) among patients with a history of unilateral cryptorchidism, and 76.9% (10/13) in those with a history of bilateral cryptorchidism.

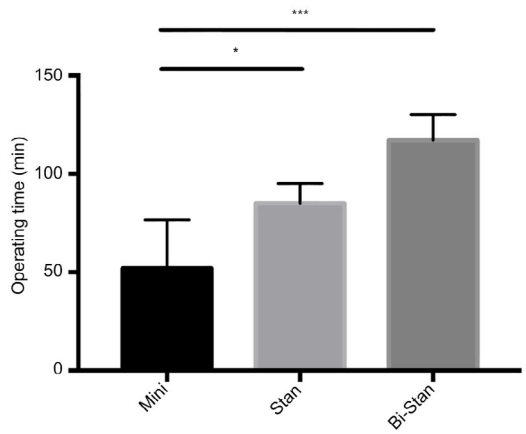

Fig. 2 Operating time for the mini-incision micro-TESE (Mini), standard micro-TESE (Stan), and bilateral standard micro-TESE (Bi-Stan) groups
Data are expressed as mean±standard deviation (SD). * $P<0.05$, *** $P<0.001$

Sperm were successfully cryopreserved for future ICSI in all cases of successful micro-TESE. In some centers, micro-TESE is usually performed on the day before oocyte retrieval or the same day to benefit from the use of fresh testicular sperm. However, this strategy may cause unnecessary ovarian stimulation and oocyte retrieval when no sperm are detected during the male procedure (Schachter-Safrai et al., 2017). Furthermore, a meta-analysis reporting on 574 cycles revealed no difference in the clinical outcomes for the use of fresh vs. frozen sperm for ICSI in men with NOA (Ohlander et al., 2014). In our institution, uncoupled micro-TESE/oocyte retrieval is performed in NOA cases to prevent possible unnecessary morbidity to the female partner. Cryopreservation also maximizes flexibility for the couple to initiate an ICSI cycle. Twelve couples attempted to initiate ICSI, two couples resorted to oocyte cryopreservation due to the absence of motile sperm post-thaw, which is the shortcoming of sperm banking. Of the remaining 10 couples, 1 couple is currently carrying an ongoing pregnancy, and 3 deliveries have occurred in other couples. A spontaneous abortion occurred in one couple.

Micro-TESE, which involves a careful dissection of the testicular tissue to minimize diminished testicular function (Schlegel, 1999), may also cause extensive tissue damage and potential long-lasting injury to the testis. To this end, we believe that optimization of sperm retrieval technique will contribute to reduced testicular injury without compromising SSR. Furthermore, due to post-surgical scarring, men with a history of prior orchidopexy may present with a challenge as the anatomy is slightly more challenging to release the testis for microscopic evaluation. The presented stepwise mini-incision micro-TESE for patients with prior orchidopexy carries the same goals as the standard micro-TESE technique: (1) to successfully harvest spermatozoa for ICSI, (2) to reduce the operating time and effort involved in surgery, and (3) to potentially minimize tissue loss without subsequent loss of testicular function. We argue that the presented "three-step" sperm retrieval method accomplishes these goals while gradually increasing surgical invasiveness (Ma et al., 2012). Our data demonstrate that half of the patients with NOA associated with a prior history orchidopexy had rapid identification of sperm in the superficial tissue,

which is less invasive and less detrimental to testicular function (Alrabeeah et al., 2016). Upon failure of the first two steps, sperm were found in the contralateral testis of one patient following progression to bilateral standard micro-TESE. Although we did not evaluate the post-operative testicular function in this cohort, we postulate that the modified stepwise micro-TESE will minimize the reduction of testicular function due to less disruption of testicular parenchyma.

Our preliminary data showed that the stepwise mini-incision micro-TESE technique could gradually increase surgical invasiveness and reduce operating time without compromising outcomes. Because of the small size of our cohort, better designed and controlled prospective studies with longer follow-up are required to help further evaluate the clinical value of the stepwise mini-incision micro-TESE for patients with a history of orchidopexy affected by NOA.

Contributors

Peng LI and Chen-cheng YAO performed the research and data analysis, wrote and edited the manuscript. Peng LI guided the surgery. Zheng LI, Peng LI, Hui-xing CHEN, Ru-hui TIAN, Zhong WAN, Ying-chuan JIANG, and Yu-hua HUANG performed the surgery. Er-lei ZHI collected the data. Chao YANG and Liang-yu ZHAO performed the sperm harvest. Yuan XU and Yue-hua GONG performed the sperm cryopreservation. Zheng LI contributed to the study design, data analysis, writing and editing of the manuscript. All authors read and approved the final manuscript and, therefore, had full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Peng LI, Chen-cheng YAO, Er-lei ZHI, Yuan XU, Zhong WAN, Ying-chuan JIANG, Yu-hua HUANG, Yue-hua GONG, Hui-xing CHEN, Ru-hui TIAN, Chao YANG, Liang-yu ZHAO, and Zheng LI declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients included in the study.

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中文概要

题　目：分步法小切口改良睾丸显微取精术在隐睾下降固定术后非梗阻性无精子症的临床应用研究

目　的：探讨分步法小切口改良睾丸显微取精术在隐睾下降固定术后非梗阻性无精子症的有效性。

创新点：既往有隐睾下降固定术手术史的非梗阻性无精子症的患者睾丸局部粘连严重，所以睾丸显微取精术时睾丸暴露困难，因此手术难度明显增大。术者将常规睾丸显微取精术改良为分步法小切口睾丸显微取精术，术中无需完全游离暴露睾丸，仅通过小切口睾丸显微取精术就可使一半的患者成功获取精子。因此分步法小切口改良睾丸显微取精术可在不降低睾丸取精成功率的前提下缩短手术时间和减少手术创伤。

方　法：收集 2015 年 5 月至 2017 年 3 月于上海交通大学附属第一人民医院泌尿中心男科接受睾丸显微取精术的 20 例既往有隐睾下降固定术手术史的非梗阻性无精子症，采用分步法小切口改良睾丸显微取精术获取睾丸精子。分步法小切口改良睾丸显微取精术具体步骤为：第一步，小切口睾丸取精术；第二步，将小切口延长为标准切口，行标准睾丸显微取精术；第三步，对侧睾丸标准睾丸显微取精术。

结　论：小切口睾丸显微取精术可快速有效获取睾丸内表浅位置饱满的生精小管，标准的睾丸显微取精术可作为小切口睾丸显微取精术失败时的补救措施。分步法小切口睾丸显微取精术可降低睾丸损伤前提下，快速有效地获取睾丸精子。这种手术方式特别适用于睾丸内存在表浅饱满生精小管的非梗阻性无精子症，如隐睾下降固定术后的非梗阻性无精子症。

关键词：非梗阻性无精子症；隐睾；睾丸显微取精术；睾丸下降固定术；精子获取