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REVIEW ARTICLE

The development of surgical sperm extraction and new challenges to improve the outcome

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Abstract Surgical sperm extraction with intracytoplasmic sperm injection has become widespread worldwide and is regarded as the sole option for patients with azoospermia. However, the sperm retrieval rate remains unsatisfactorily low, particularly for men with non-obstructive azoospermia (NOA). Therefore, the technical challenges associated with improving the sperm retrieval rate for men with NOA are being addressed. The most successful method developed to date is microdissection testicular sperm extraction (micro-TESE), which is rapidly becoming recognized as a useful technique due to its relatively high sperm retrieval rate and low complication rate. However, even with micro-TESE, the sperm retrieval rate for men with NOA remains at 30–60 %, with an even lower birth rate. The technical challenges associated with improving the outcomes of surgical sperm extraction are being approached through the use of ultrasound and optimal surgical devices such as narrow band imaging, multiphoton microscopy, and optical coherent tomography. In addition to the difficulties related to searching for sperm, medical treatments that induce spermatogenesis remain controversial. For example, varicocele repair prior to surgical sperm extraction and hormonal therapy before and after TESE have been extensively examined. We herein briefly summarized the development process in surgical sperm extraction up to the present and technical challenges to improve the outcomes of surgical sperm extraction.

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Introduction

Azoospermia, defined as the absence of spermatozoa in semen, is the most serious condition influencing the ability of men to reproduce, and has been reported in 1 % of the general population and in 10–15 % of infertile men [1, 2]. The first attempt to achieve fertilization by men with obstructive azoospermia (OA) was reported in 1985, and used sperm obtained from epididymal spermatozoa and in vitro fertilization (IVF) [3, 4]. However, this procedure was not popular due to the low successful pregnancy rate, until the introduction of intracytoplasmic sperm injection (ICSI) in 1993 [5, 6]. ICSI has led to advances in the ability of male patients with primary infertility to achieve fertilization. The first pregnancies after fertilization by ICSI with testicular sperm from men with OA were reported in 1993 [7–9], and were unexpected because they demonstrated that it was possible to bypass epididymal factors such as glycoproteins using artificial reproductive technology (ART). The application of testicular sperm extraction (TESE) to men with non-obstructive azoospermia (NOA) and resultant pregnancies were reported in 1995 [10, 11]. However, spermatogenesis is limited in men with NOA, with TESE failing in approximately 60 % of cases [12–15]. Therefore, the technical challenges associated with improving the successful sperm retrieval rate are being addressed. TESE with multiple biopsies was previously reported to improve the sperm retrieval rate because sperm are only obtainable from isolated regions in many cases of NOA, and the possibility of detecting spermatozoa was not influenced by location in the testis [16–18]. However, TESE with multiple biopsies may result in the loss of a significant amount of testicular tissue and interrupt the testicular blood supply, which may ultimately lead to testicular atrophy [19, 20]. On the other hand, several groups have used fine needle aspiration for sperm retrieval as a minimally invasive technique [21]. However, due to the limited number of spermatozoa retrieved, the sperm retrieval rate in NOA patients remains insufficiently low [22]. In 1999, microdissection (micro)-TESE was reported for the first time as a technique to achieve a high sperm retrieval rate and low complication rate through the identification of spermatogenically active regions during surgery [23]. A large number of studies have reported that the outcomes of micro-TESE are more favorable than those of conventional single or multiple TESE, such that it is now regarded as the first-line treatment for men with NOA [24–26]. However, the successful sperm retrieval rates by micro-TESE in men with NOA remain unsatisfactory, ranging between 30 and 60 % [24, 25, 27–29]. Furthermore, micro-TESE requires a significant learning curve and long operative time [26, 27]. Therefore, this surgical procedure needs to be improved in order to increase the sperm retrieval rate and shorten the learning curve.

In addition to the challenges related to the search for sperm, medical treatments to induce spermatogenesis remain controversial. For example, varicocelectomy for NOA patients and hormonal therapy have been extensively examined.

The aim of this review was to discuss the advantages and disadvantages of existing sperm retrieval techniques and some of the technical challenges associated with improving the outcomes of surgical sperm extraction for men with NOA.

Evaluation points

The goal of surgical sperm retrieval is to obtain an adequate number of sperm for ART and minimize damage due to the surgical procedure. Although the evaluation of live birth rates is desirable, we herein mainly evaluated the sperm retrieval rate because the successful outcome of ICSI measured by live birth rates was previously reported to be independent of the type of procedure [30]. Tissue damage was assessed by hormonal changes after the surgical procedure and the complication rate.

Testicular sperm aspiration

Testicular sperm aspiration (TESA) was developed from the testicular biopsy technique. The main advantage of TESA is its simplicity and safety, because it does not require surgical equipment or expertise. Several methods have been described for fine needle aspiration (FNA) to obtain spermatozoa: a biopsy gun or 19-gauge to 23-gauge needle [13, 31–33]. When a 21-gauge or fine needle is used, it is possible to performed aspiration without anesthesia and the complication rate is lower than that of all other methods. However, Rosenlund et al. reported that the sperm retrieval rate using a 21-gauge needle was markedly lower than that using a 19-gauge needle (11 vs 62 %), and this was attributed to the retrieval of more tissue with a larger needle, resulting in a greater yield of spermatozoa [34]. As shown in Table 1, the sperm retrieval rate of FNA is lower than that of TESE [13, 34–40]. Therefore, the acquisition of more tissue increases the possibility of obtaining sperm. On the other side, the risk of complications has been related to the volume of tissue removed. Haggar found that the complication rate of TESA was 22 % higher than that of previous findings, and attributed this to the larger number of puncture sites (an average of 12–18 punctures). As described previously, the main advantages of TESA over TESE is its simplicity and safety. Therefore, the high complication rate and long operative time due to multiple biopsies may offset the advantages of TESA. Moreover, TESE and micro-TESE have recently and safely been performed under local anesthesia with a cord block, which had previously been considered to require general anesthesia. Therefore, TESA is inferior to TESE and micro-TESE due to its lower sperm retrieval rate, and has only a few advantages because of its safety and simplicity.

TESE

The use of testicular sperm was initially introduced as an alternative to epididymal sperm (percutaneous epididymal sperm aspiration: PESA, microsurgical epididymal sperm aspiration: MESA), and the first pregnancies after fertilization by ICSI using testicular sperm from men with OA were reported in 1993 [7–9]. This was unexpected, because it demonstrated that it was possible to bypass epididymal factors such as glycoproteins by ART. Testicular spermatozoa from men with NOA have since been shown to possess the ability to fertilize oocytes and produce viable embryos [11, 12, 14]. Nicopoullos found that ICSI outcomes in a meta-analysis were not affected by the sperm source (epididymal or testicular), fertilization rate (RR 1.01; 95 % CI 0.97–1.18; $P = 0.16$), clinical pregnancy rate (RR 1.01; 95 % CI 0.85–1.25; $P = 0.94$), or live birth rate (RR 0.98; 95 % CI 0.71–1.36; $P = 0.91$) [30]. TESE was, until recently, considered the gold standard for retrieving spermatozoa from men with NOA due to the provision of more tissue than TESA or MESA and the

Table 1 Comparison of the outcome in testicular sperm aspiration (TESA) and testicular sperm extraction (TESE)

Year	Author	Testicular sperm aspiration (TESA)				Testicular sperm extraction (TESE)		
		Case (*n*)	Needle (gauge)	Sperm retrieval rate (%)	Complication	Case (*n*)	Sperm retrieval rate (%)	Complication
1997	Friedler	37	21	11	2.7 % of bleeding	37	43	2.7 % of hematoma
1998	Rosenlund	17	19	12	0	17	47	0
		16	21	63	0	16	75	0
1999	Ezeh	35	19	14		35	63	
1999	Lewin	85	23	58.8	3.5 % of pain			
				48.3 (SCO)				
				46.4 (MA)				
				95 (HS)				
				66.6 (TH)				
2001	Nassar	49		24.5			26	19.2
2003	Aridogen	38	26	39.5		38	40.8	
2006	Hauser	87	18	24.1		87	62.1	
				16.7 (SCO)			33.3 (SCO)	
				37.5 (MA)			45.8 (MA)	
				69.0 (HS)			100 (HS)	
2007	Haggar	100	23	10	14 % of intratesticular hematoma	100	52	
		40		5 (SCO)	6 % of skin bruises	40	30 (SCO)	4 % of intratesticular hematoma
		12		16.7 (MA)	2 % of hematocele	12	16.7 (MA)	2 % of intratesticular oedema
		34		18 (HS)		34	100 (HS)	
		14		0 (TH)		14	28.6 (TH)	

SRR sperm retrieval rate, *SCO* Sertoli cell only, *MA* maturation arrest, *HS* hypospermatogenesis, *TH* tubular hyalization

higher possibility for sperm retrieval. However, although the sperm retrieval rate of TESE is higher than that of TESA or MESA, it remains at approximately 20–60 % for NOA patients, which is unsatisfactory [41, 42]. Therefore, the technical challenges associated with improving the sperm retrieval rate are still being addressed. The most well-known attempt is multiple biopsies, which is based on the theory that the NOA testis has an uneven distribution of regions with minimal spermatogenesis [18, 43, 44]. Hauser suggested that the performance of multiple testicular biopsies increased the probability of detecting spermatozoa in cases of NOA (28.6 % by one biopsy, 53.6 % by three biopsies) [18]. Hauser also showed that the ability to locate spermatozoa was not influenced by location in the testis, which indicated that not only the amount of tissue, but also variations in the biopsy site, were related to improvements in the sperm retrieval rate. On the other hand, Witt et al. reported that the highest sperm retrieval rate was obtained in the midline portion of the testis [45]. The optimal number of biopsies and most suitable sites in the testis remain controversial. Therefore, more studies are needed in order to apply these findings to clinical settings; however, the prevalence of micro-TESE has detracted attention from clinical research on TESE.

Micro-TESE

Micro-TESE was first described in 1998 [46, 47] and has become the most frequently used method for sperm retrieval from men with NOA. Micro-TESE is an advanced version of TESE that employs microsurgical techniques to identify individual seminiferous tubules that are most likely to contain sperm. Schlegel indicated that multiple testicular biopsies with multiple incisions in the tunica albuginea interrupted the testicular vasculature [19]. Therefore, multiple testicular biopsies need to be avoided in order to reduce the risk of testicular atrophy. On the other hand, micro-TESE has the ability to identify the vascular structure at the opening of the tunica albuginea and minimize the incidence of vascular injury.

Many studies have compared the sperm retrieval rates of micro-TESE and conventional TESE. As shown in Table 2, the sperm retrieval rate of micro-TESE is higher than that of conventional TESE [23–26, 48, 49]. A recent meta-analysis of 1890 NOA patients revealed that, in a direct comparison, the performance of micro-TESE was 1.5-fold more likely to result in successful sperm retrieval than conventional TESE [50]. This tendency is more remarkable in histological patterns of patchy spermatogenesis such as Sertoli-cell-only syndrome [42]. Although the sperm retrieval rate of micro-TESE is higher than that of conventional TESE or TESA, a universal consensus has not yet been reached regarding micro-TESE being the only method of sperm retrieval for NOA patients. Specifically, for example, the sperm retrieval rates of TESE are different among the comparison groups in the previously described meta-analysis, suggesting that they are unlikely to be accurate (micro-TESE vs. TESE; 52 vs. 35 %, TESA vs. TESE; 28 vs. 56 %) [50]. This may be due to differences in the patient population, surgeon experience, embryologist experience, and selection bias of the sperm retrieval procedure. Therefore, the actual sperm retrieval rates of these procedures still remain unknown. Moreover, micro-TESE has some disadvantages; for example, the long operative time and significant learning curve [27]. Therefore, larger studies with standardized reporting are required in order to obtain a better understanding of differences in the benefits of each technique, as well as to identify the most appropriate procedure for each case.

Developing techniques for TESE

The technical challenges associated with improving sperm retrieval from testes have been examined in detail. The use of Doppler ultrasound is a well-known technique for TESE in men with NOA, and is based on the theory that the testicular structure of men with NOA has isolated sites of spermatogenesis. Furthermore, it has been suggested that the foci of spermatogenesis in men with NOA are located in regions with abundant blood perfusion [51]. Based on these findings, Har-Toov et al. reported that power Doppler ultrasound was useful for predicting the presence of spermatozoa in testicular biopsies [52]. Herwig et al. also applied laser Doppler scanning to measure tissue perfusion in patients undergoing TESE, and demonstrated that tissue perfusion mapping was useful for improving the outcomes of TESE; sperm quality and quantity were found to be dependent on tissue perfusion [53, 54]. Perfusion-controlled testicular biopsy may allow for predictable TESE and appears to be superior to random TESE. The main limitation of this technique is the difficulty associated with obtaining tissue when Doppler ultrasound identifies a well-perfused area close to the center of the testis because the TESE incision is small. In order to overcome this issue, we recently reported a new technique combining micro-TESE and the monitoring of tissue perfusion with narrow band imaging (NBI) [55]. The NBI system uses blue narrow band light (390–445 nm) to visualize capillaries in the surface layers of mucosal membranes, and green narrow band light (530–550 nm) to visualize thick blood vessels located inside membranes while enhancing the contrast of surface capillaries [56]. Using this system, small capillaries are observed as brown and thick vessels as green. We applied these characteristics to the testis and found that NBI had the ability to identify spermatogenically active regions by color in a rodent model [55]. Similar techniques for sperm extraction have been reported using optical systems other than surgical microscopy. For example, Ramasamy et al. reported that multiphoton microscopy (MPM) identified the stage of spermatogenesis in a rodent model [57]. MPM uses pulsed long-wavelength light that penetrates tissue in order to excite fluorophores within the specimen being observed. Therefore, according to Ramasamy et al. MPM has the ability to identify normal spermatogenesis as well as derangements in spermatogenesis in the cellular architecture of the seminiferous tubules. The use of full-field optical coherent tomography (FFOCT) has also been reported by Ramasamy et al. [58]. FFOCT is based on the theory of the interferometric selection of single back-scattered photons using the superposition of

Table 2 Comparison of the outcome in conventional testicular sperm extraction (conventional-TESE) and microdissection testicular sperm extraction (Micro-TESE)

Year	Author	Conventional-TESE			Micro-TESE		
		Case (*n*)	SRR (%)	Complication	Case (*n*)	SRR (%)	Complication
1999	Schlegel	22	45	0	27	63	0
2000	Amer	100	30	51.7 % hematoma 30 % fibrosis	100	47	6.7 % hematoma 3.3 % fibrosis
2002	Okada	24	16.7	7.5 % hematoma	74	44.6	2.5 % hematoma
2002	Tsujimura	37	35.1	0	56	42.9	0
2005	Ramasamy	83	32		460	57	
2011	Ghalayini	68	38.2	0	65	56.9	0

Adapted with permission from Ref. [42]

SRR sperm retrieval rate

waves to decipher their characteristics. Therefore, FFOCT is used as a high-resolution "optical biopsy" technique that quickly renders images of freshly excised tissue [59]. Using this technology, Ramasamy et al. identified spermatogenesis within the seminiferous tubules in freshly excised testicular tissue without exogenous contrast or fixation in a rodent model [58].

Although these techniques are still in the experimental stage, they have the potential to improve the success rate of sperm extraction from patients with NOA and reduce the number of biopsies due to the estimation of spermatogenically active regions in the testis, which minimizes testicular damage and surgical times.

Varicocele repair before TESE

Approximately 15 % of male infertility cases have been attributed to varicocele [60]. Varicocele repair has been reported to improve spermatogenesis in the testes of men with NOA and varicocele, leading to the return of sperm in the ejaculate in 22–55 % of these men and the avoidance of TESE in 10–40 % [61, 62]. On the other hand, Schlegel et al. found that men with NOA associated with clinical varicocele rarely had adequate amounts of sperm in the ejaculate after undergoing varicocelectomy to an attempt to avoid TESE [63]. Table 3 shows the outcomes of varicocelectomy in men with NOA in previous studies [61, 64–78]. These findings indicate that a certain percentage of men with NOA and varicocele successfully recovered sperm in the ejaculate after varicocelectomy, and the recovery of sperm appears to be dependent on testicular histology. Therefore, even if sperm does not reappear in the ejaculate, the sperm retrieval rate in NOA men post-varicocelectomy may be better than in those who do not undergo this procedure. However, few studies have examined the effects of varicocelectomy on the outcomes of TESE. Schlegel and Kaufmann evaluated 138 NOA patients with a history of varicocele who underwent TESE. Of these, 68 patients underwent varicocelectomy for varicocele, whereas 70 did not. The findings of that study revealed no significant difference in the sperm retrieval rate between these two groups (60 % in patients who underwent varicocelectomy, 60 % in those who did not). This finding was similar at every stage of histopathology (SCO 26 and 38 %, MA 53 and 47 %, HS 96 and 96 % in the post-varicocelectomy group and without varicocelectomy group, respectively) [63]. In contrast, Inci et al. reported that the sperm retrieval rate of TESE in NOA patients with varicocele was higher in the post-varicocelectomy group (53 %) than in the without varicocelectomy group (30 %) [79]. However, this study was a chart review retrospective study and no data was provided on histopathology before varicocelectomy. Therefore, the effects of varicocelectomy on the testes of NOA patients remain unknown.

Another concern is the length of time needed for sperm to appear in the ejaculate after varicocele repair. Since the entire process of spermatogenesis takes 74 days, at least 3 months is required for sperm recovery. We previously reported that the total motile sperm count improved 3 months after varicocelectomy and did not subsequently change until 12 months after varicocelectomy [80]. Pasqualotto et al. showed that nine out of 27 NOA patients who underwent varicocelectomy had sperm in their ejaculate at 6 months post-varicocelectomy; however, five patients (55.6 %) returned to azoospermia at 12 months post-varicocelectomy, demonstrating that the effects of varicocelectomy may be temporary. Therefore, these findings suggest that the time period required for the appearance of sperm is between 3 and 9 months after varicocelectomy, and cryopreservation needs to be considered when sperm appear in the ejaculate.

In conclusion, although some disadvantages are associated with this rapid treatment, varicocele repair prior to TESE represents a reasonable option for men with NOA, particularly younger couples, because some patients, if not most, may achieve some benefit from varicocelectomy.

Hormonal therapy before or after TESE

Hormonal treatments for NOA are considered to be ineffective because of high gonadotropin levels; however, previous studies reported that anti-estrogens, aromatase inhibitors, and gonadotropins stimulated spermatogenesis [81]. Ramasamy et al. administered a pretreatment with aromatase inhibitors for patients with Klinefelter's syndrome whose serum testosterone levels were less than 300 ng/dl prior to micro-TESE. Among their cohort of 68 men, testicular spermatozoa were successfully retrieved in 45 men (66 %), and the pretreatment did not have a significant impact on successful outcomes. However, they noted that patients who required and responded to the pretreatment with a resultant testosterone level of 250 ng/dl or higher had better outcomes [82].

Anti-estrogen represented by clomiphene citrate is the most popular choice as hormonal therapy for NOA due to its safety and simplicity. Hussein et al. administered a treatment with clomiphene citrate to men with NOA with testicular results of hypospermatogenesis or maturation arrest. They found that 64.3 % of men had sperm in their ejaculate within 6 months of the treatment, and a sufficient amount of sperm was successfully retrieved in men who remained azoospermic using micro-TESE [83]. These findings indicate that surgeons may consider a course of clomiphene citrate prior to surgical TESE. However, men

Table 3 Outcome of the varicocele repair in men with non-obstructive azoospermia

Year	Author	Case (*n*)	Appearance of sperm to ejaculate			
			Overall (%)	SCO (%)	MA (%)	HS (%)
1998	Matthews	22	12 (55)			
1999	Kim	28	12 (43)	0/3 (0)	5/13 (38)	9/18 (50)
2001	Kadioglu	24	5 (21)	3/7 (43)	1/14 (7)	1/3 (33)
2004	Schlegel	31	7 (22)			
2004	Caken	13	3 (23)	0/5 (0)	0/3 (0)	3/5 (60)
2005	Esteves	17	8 (47)	0/6 (0)	3/5 (60)	5/6 (83)
2005	Gat	32	18 (56)			
2006	Poulakis	14	7 (50)			
2006	Pasqualotto	27	9 (33)	4/10 (40)	3/8 (38)	2/9 (22)
2007	Lee	19	7 (36)	1/10 (10)	4/6 (67)	2/3 (67)
2008	Ishikawa	6	2 (33)			
2009	Cocuzza	10	3 (30)	1/4 (25)	0/4 (0)	2/2 (100)
2009	Youssef	51	14 (28)	2/22 (9)	6/26 (23)	6/23 (26)
2012	Abdel-Meguid	31	10 (32)	0/10 (0)	3/8 (38)	7/13 (54)
Overall		325	117 (36)	11/77 (14)	25/89 (28)	37/82 (45)

Adapted with permission from Ref. [78]

SCO sertoli cell only, *MA* maturation arrest, *HS* hypospermatogenesis, *TH* tubular hyalization

with SCO or a low testicular volume and a mean serum FSH at baseline of 7.21 mIU/ml were excluded from that study; therefore, the efficacy of clomiphene citrate remains unknown for men with NOA.

The administration of gonadotropins to men with NOA is a more comprehensive therapy typically used in combination with hCG and recombinant FSH [81]. Shiraishi et al. reviewed 48 men with NOA who had negative sperm retrieval results by micro-TESE. Second micro-TESE was subsequently performed on these men: 20 were not treated with any hormonal therapy, while 28 received daily injections of hCG with additional FSH if endogenous gonadotropin levels decreased during the hCG stimulation. The second TESE was successful in 21 % of the 28 patients who received hormonal therapy, whereas it failed in all patients who did not receive hormonal therapy [84]. They also reported that the histological pattern in the first micro-TESE correlated with the outcomes of second TESE (success rates of 50 % with HS, 25 % with MA, and 0 % with SCO). Selman et al. evaluated a more selective group of men with normal baseline hormone profiles, testicular volumes, and a genetic analysis resulting in the first histological pattern of MA. Among the 49 patients examined, 11 (22.4 %) had sperm in their ejaculate by the second TESE following the FSH treatment [85].

Difficulties associated with these treatments include the low success rate of sperm retrieval and even lower pregnancy rate [85], as well as treatment costs. Larger studies are needed in order to identify appropriate candidates for hormonal therapy and the optimal doses of medication used.

Conclusion

Among the sperm retrieval techniques available for men with NOA, TESE is superior to TESA and micro-TESE is superior to TESE due to higher sperm retrieval rates and lower complication rates. However, since a prospective randomized controlled trial study has not yet been conducted, no definitive conclusions can be reached. New directions to improve the outcomes of surgical sperm extraction have been investigated, such as the combined use of ultrasound, NBI, MPM, and OCT. Although these techniques are still in the experimental stage, they have the potential to improve the success rate of sperm extraction for men with NOA.

Other attempts, in addition to the development of searching techniques, include medical therapy to induce spermatogenesis such as varicocele repair before TESE and hormonal therapy. Of these, varicocele repair prior to TESE may induce spermatogenesis in some patients, and is regarded as a reasonable option for men with NOA, particularly for younger couples. Hormonal therapy for men with NOA is another popular treatment to induce spermatogenesis. Although positive findings have been reported for hormonal therapy such as anti-estrogens, aromatase inhibitors, and gonadotropins, evidence for their use as a standard therapy is still insufficient. Further studies are needed in order to establish more appropriate treatment regimens for each patient.

Compliance with ethical standards

Conflict of interest Noritoshi Enatsu, Koji Chiba, Masato Fujisawa declare that they have no conflict of interest.

Human/animal rights This article does not contain any studies with human or animal subjects performed by any of the authors.

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