—Mini Review—

Testicular sperm extraction and varicocelectomy for severe male infertility

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Abstract: Varicocele repair and testicular sperm extraction (TESE) are most commonly performed for infertile males. Generally, TESE is performed to retrieve spermatozoa in azoospermic patients, and varicocelectomy is used to restore the possibility of spontaneous pregnancy by recovering sperm parameters. Most recently, indications for these treatments have been extended with the spread of intracytoplasmic sperm injection (ICSI). To decrease the DNA damage of spermatozoa, TESE for severe oligozoospermia and varicocelectomy for azoospermia or severe oligozoospermia are considered treatment options. However, there is insufficient data with which to draw conclusions about the efficacy of these treatments; the outcomes appear to show improvement to some extent. To achieve a successful ICSI outcome for the improvement of male factor infertilities, these treatments should be offered to selected patients. During selection, patients, especially those with severe oligozoospermia who frequently have a genetic disorder should undergo genetic analysis before treatment.

Key word: Male infertility, TESE, Varicocele, Severe oligozoospermia, Sperm DNA damage

Introduction

Approximately 50% of infertile couples are affected by male factor infertility [1]. Therefore, male infertility must be addressed when considering the outcome of reproduction treatment. To improve the results of reproductive treatment from the aspect of male factor infertility, we have a goal of achieving improvements in the quality and quantity of spermatozoa. Two major treatments, varicocele repair and testicular sperm extraction (TESE) play key roles in improving the infertility of subfertile males. Sperm DNA damage decreases the quality of spermatozoa and is increased in infertile men with varicoceles, and high levels of damage are associated with a decreased pregnancy rate, including that of spontaneous pregnancy [2, 3]. Varicocele repair has also been suggested as a treatment for reducing sperm DNA damage, and low DNA damage is associated with a higher pregnancy rate [4]. TESE is used exclusively to treat for non-obstructive azoospermia (NOA) in order to increase the chance of pregnancy. Microdissection testicular sperm extraction (micro-TESE) using an operating microscope was first reported in 1999 [5], and this procedure can minimize the risk of damage to the testicular blood supply as well as increase the sperm retrieval rate [6]. Micro-TESE has become an established procedure for NOA patients over the last decade. Because the outcome of ICSI in cases with very low sperm count is poorer than that of patients with mild oligozoospermia [7], the indications for micro-TESE have recently been extended to include patients with severe oligozoospermia or cryptozoospermia, with the aim of reducing sperm DNA damage.

In this review, we address varicocelectomy and TESE in terms of the probability of reducing spermatozoa damage and improving the outcome of reproductive treatment.

Varicocele

Varicocele and male infertility

Varicoceles are defined as abnormally dilated scrotal veins. They are the most common cause of male infertility and are present in 20% of the normal male population and in approximately 40% of men presenting with infertility [8]. According to some studies regarding the pathophysiology of varicocele, several causes of impaired spermatogenesis are proposed, including endocrine disturbances, heat stress, oxidative stress, and autoimmune abnormalities [9–12].

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Diagnosis and grading

The patient should be examined in the standing position, both before and after a Valsalva maneuver. Palpation of the pampiniform plexus is performed and then varicocele grading is assessed as follows:

1. Grade I - palpable dilated veins only after a Valsalva maneuver.

2. Grade II - palpable dilated veins in the standing position without a Valsalva maneuver, yet not visible.

3. Grade III - clearly visible dilated veins in the standing position without a Valsalva maneuver.

Scrotal color doppler ultrasound is useful as an adjunct to the diagnosis of varicocele to assess the diameters of dilated veins and visualize the reflux venous flow. Although definitive criteria have not been established, the presence of two or more veins with diameters larger than 3.0 mm is consistent with a diagnosis of clinical varicocele [13]. Varicocles that are not palpable in a physical examination but can be identified by scrotal ultrasound are defined as subclinical varicocles, and are not considered significant.

Indications for treatment of a varicocele

In the last two decades, numerous studies have reported the efficacy of varicocele treatment for subfertile couples. Many of these studies included men with a subclinical varicocele and normal semen parameters. As a result, a systematic review concluded the impact of varicocele repair is not effective [14]. However, three recent meta-analyses have shown that varicocele repair significantly improves sperm concentration and motility in infertile men with a clinical varicocele and abnormal preoperative semen parameters [15–17]. Therefore, common indications for varicocele repair in adult men include couple infertility and symptomatic varicoceles. Based on The Practice Committee of the American Society for Reproductive Medicine [18], varicocele treatment should be offered when the following conditions are met: 1) the varicocele is palpable in a physical examination of the scrotum; 2) the couple has known infertility; 3) the female partner has normal fertility or a potentially treatable cause of infertility; and 4) the male partner has abnormal semen parameters or abnormal results in sperm function tests. Varicocele repair is not indicated for men with normal semen analyses or with a subclinical varicocele.

Outcome of varicocelectomy

A recent systematic review [19] and a meta-analysis [17] reported higher spontaneous pregnancy rates after varicocele repair, and the odds ratios (ORs) were 1.47 and 2.23, respectively. Kroese *et al.* [19] analyzed 10 studies among which, five studies included men with normal semen parameters and a subclinical varicocele. They conducted subgroup analysis using the data of the five studies including only the men with abnormal semen analysis results and a clinical varicocele. The results of the subgroup analysis favored treatment with an OR of 2.39, similar to the results of Baazeem *et al.* [17]. Both studies concluded that varicocelectomy is moderately superior to observation, but the effects were not statistically significant because the studies included in the meta-analyses had considerable heterogeneity. However, Baazeem *et al.* also concluded that varicocele repair has a significant beneficial effect on sperm concentration as well as total and progressive motility.

Varicocelectomy for severe oligozoospermic or cryptozoospermic patients

According to WHO nomenclature, cryptozoospermia is defined as the condition when spermatozoa are not observed in a fresh semen sample but are observed in a centrifuged pellet [20]. The efficacy of varicocelectomy for patients with severely impaired spermatogenesis including severe oligozoospermia and cryptozoospermia is still unclear. Kamal *et al.* [21] and Matkov *et al.* [22] studied the effect of varicocelectomy on the semen parameters and pregnancy rates of clinical varicocele patients. Both of study groups stratified the patients according to their sperm concentrations and compared the outcomes, and both concluded that varicocelectomy significantly increased the semen parameters and pregnancy rates in the overall population, although the treatment was less effective for men with low sperm concentrations. Kamal *et al.* proposed that couples with sperm concentrations <5 million/ml should consider assisted reproduction (e.g., IVF/ICSI) as an alternative to varicocelectomy, because of its lower spontaneous pregnancy rate. Matkov *et al.* also suggested that although patients with total motile sperm <5 million are better initial candidates for assisted reproduction, while noting that varicocelectomy is the most cost-effective initial intervention for males with total motile sperm >5 million. Table 1 summarizes the outcomes of varicocele repair for the patients with severe oligozoospermia. Spontaneous pregnancy rates in post varicocele therapies were 8–20% and unassisted pregnancy rates including spontaneous pregnancy and intrauterine insemination (IUI) were 20–29.3%. These were all retrospective studies; therefore, it is difficult to assess whether varicocele repairs are effective or not. However, considering that infertile couples with severe male factor infertility can achieve pregnancy without IVF/ICSI, varicocele treatment may be offered to selected infertile

Table 1. The outcomes of varicocele repair for the patients with severe oligozoospermia

	Kamal *et al.* [21]		Matkov *et al.* [22]		Ghanem *et al.* [24]		Enatsu *et al.* [23]	
	Before therapy	After therapy	Before therapy	After therapy	Before therapy	After therapy	Before therapy	After therapy
Sperm concentration (million/ml)	3.6 ± 0.7	8.0 ± 2.5	1.8	12.3	2.2 ± 0.2	6.5 ± 0.5	2.4 ± 1.5	11.6 ± 1.8
Sperm motility (%)	8.1 ± 1.5	23.7 ± 3.9	N.A	N.A	13.6 ± 0.9	32.4 ± 1.2	32.8 ± 18.6	42.2 ± 2.5
Total pregnancy rate (%)		30.0		60.0		59.3		N.A
Spontaneous (%)		8.0		20.0		15.0		17.6
IUI (%)		12.0		5.0		14.3		N.A
IVF/ICSI (%)		10.0		35.0		30.0		N.A

IUI, intrauterine insemination; IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection.

couples. Matkov *et al.* and Enats *et al.* [23] classified patients into a responder group and a non-responder group according to the post-operative sperm concentration. Interestingly, pregnancy rates in the non-responder group were lower than those in the responder group. Nevertheless, some patients in the non-responder group achieved spontaneous pregnancy, and the rate was 3.3%. This indicates that varicocele treatment may recover sperm DNA damage without significantly increasing semen parameters in some cases.

In summary, varicocele repair can increase the pregnancy rate of infertile couples even when male partners have severely impaired spermatogenesis, although the effect of the treatment is greater in patients with mild or moderate oligozoospermia. While spontaneous pregnancy is expected in few cases, varicocele treatment can increase the rate of pregnancy through artificial reproduction by decreasing DNA fragmentation [4].

Varicocelectomy for azoospermic patients

Is TESE/ICSI the only intervention for azoospermic men with a clinical varicocele? The efficacy of varicocelectomy for NOA patients needs to be quantified.

Tulloch *et al.* reported on the result of varicocelectomy for 30 subfertile males in 1955 [25]. This study included three NOA patients among whom spermatozoa were found in the ejaculates of two, and these two patients achieved spontaneous pregnancy nine months after the operation. After publication of that article, several studies described varicocelectomy for azoospermic patients; however, no randomized control study has been reported, and only studies with the small sample sizes have been reported.

Pasqualotto *et al.* [26] reported the results of microsurgical varicocelectomy for NOA patients. Nine out of 27 (33.3%) had spermatozoa return to the ejaculate after varicocelectomy. One out of 9 (11.1%) patients achieved an unassisted pregnancy. All the patients underwent testicular biopsy and germ cell aplasia was identified in 10, hypospermatogenesis (HS) in nine, and maturation arrest (MA) in eight. Only 12 out of 27 underwent karyotyping and Y chromosome microdeletion analysis.

Lee *et al.* [27] reported 19 NOA patients underwent microsurgical varicocelectomy and testicular biopsy. All the patients had a normal karyotype and no Y chromosome microdeletions. Motile spermatozoa in the ejaculate were identified in seven patients (36.4%) after varicocelectomy. However, 2 out of these 7 patients with motile spermatozoa had returned to an azoospermic state at their second postoperative semen analysis. Among the 7 patients, 2 had HS, 4 had MA, and 1 had Sertoli cell-only syndrome (SCO). Spontaneous pregnancy was achieved in 1 out of the 19 (5.3%) with HS.

Cocuzza *et al.* [28] reported on 10 NOA patients with a clinical varicocele (only grade2–3) who underwent testicular biopsy and microsurgical varicocelectomy using an inguinal or subinguinal approach. Patients with chromosomal abnormalities and Y chromosome microdeletions were excluded. Testicular biopsy revealed 3 patients had HS, 4 had MA, 3 had SCO. Two patients with HS and 1 patient with MA had spermatozoa in the ejaculate after the operation. The sperm concentration of the patient with MA recovered to 12.2 million/ml, surprisingly. However, there was no report of pregnancy in this article.

Abdel *et al.* [29] reported a prospective uncontrolled study including NOA patients with palpable clinical varicoceles. The patients underwent simultaneous subinguinal microsurgical varicocelectomy and testicular biopsies, and their semen was analyzed at 3 and 6 months after the operations. Motile spermatozoa recovery was evident in 10 out of 31 (32.3%) patients ; 7 out of 13, 53.8% with HS and 3 out of 6, 50% with late MA. However, no sperm could be recovered from the ejaculate of patients with early MA or SCO. Among the 10 cases of motile spermatozoa recovery, 6 patients achieved persistent sperm recovery, 2 showed intermittent sperm re-

Table 2. Comparison of sperm retrieval rates of TESE

	Sperm retrieval rates	
	TESE with preceding varicocelectomy	TESE without preceding varicocelectomy
Zampieri *et al.* [30]	57.8% (11/19)	25% (4/16)
Haydardedeoglu *et al.* [31]	60.8% (45/74)	38.5% (75/195)
Inci *et al.* [32]	53% (35/66)	30% (9/30)

TESE, testicular sperm extraction.

Table 3. Rates of sperm recovery in the ejaculate and spontaenous pregnancy after varicocele repair

	Spermatozoa in the ejaculate				Spontaneous pregnancy
	HS	MA	SCO	Total	
Pasqualotto *et al.* [26]	NA	50% (1/2)	36.3% (4/11)	38.4% (5/13)	0% (0/13)
Lee *et al.* [27]	66.7% (2/3)	66.7% (4/6)	10% (1/10)	36.8% (7/19)	5.3% (1/19)
Cocuzza *et al.* [28]	100% (2/2)	25% (1/4)	0% (0/4)	30% (3/9)	NA
Abdel-Meguid [29]	53.8% (7/13)	37.5% (3/8)	0% (0/10)	32.2% (10/31)	NA
Zampieri [30]	NA	NA	NA	48.6% (17/35)	0% (0/35)

HS, hypospermatogenesis; MA, maturation arrest; SCO, Sertoli cell only; NA, not available.

covery, and the remaining 2 relapsed into complete azoospermia. This result is important in the consideration of therapeutic strategies for maximizing the limited opportunity after varicocelectomy for NOA patients.

Zampieri *et al.* [30] reported an observational study including NOA patients with only grade 3 varicoceles. At 6 months after microsurgical varicocelectomy, semen analyses of all the patients revealed that 17 out of 35 (48.6%) patients had spermatozoa in the ejaculate, but none of them achieved a spontaneous pregnancy. Zampieri *et al.* also reported the sperm retrieval rate of TESE after varicocelectomy. The NOA patients with grade 3 varicocele were treated with 2 different strategies according to the timing of varicocelectomy: Group 1 underwent TESE 3 months after varicocelectomy, whereas Group 2 underwent TESE at the time of varicocelectomy. The sperm retrieval rate of TESE was significantly ($P < 0.05$) greater in Group 1 (57.8%, 11/19) than in Group 2 (25%, 4/16; Table 2).

Haydardedeoglu *et al.* [31] and Inci *et al.* [32] also reported a higher sperm retrieval rate of TESE for NOA after varicocelectomy than TESE for NOA without varicocelectomy (Table 2). However, there were no significant differences in the clinical pregnancy and live birth rates between the treated and untreated groups of either study.

In summary, varicocele repair can enable the retrieval of motile sperm from the ejaculate of NOA patients with a clinical varicocele. Table 3 shows the results of the studies which indicate NOA patients with clinical varicocele frequently had HS and MA, and these histological patterns were positive predictive factors for sperm recovery from the ejaculate after varicocele repair. Although total rates of sperm recovery in the ejaculate were 30–48.6%, spontaneous pregnancy rates were very low (0–5.3%). Therefore, we should not be excessively hopeful for pregnancy without assisted reproductive treatment, even when spermatozoa are found in the ejaculate after varicocele treatment. Furthermore, if motile spermatozoa cannot be recovered from the ejaculate, varicocele repair may allow spermatozoa to be retrieved by TESE (Table 2). In summary, varicocele repair may be effective for NOA patients with a clinical varicocele. Therefore, varicocele repair should be performed for NOA patients when the treatment can be conducted immediately.

TESE for cryptozoospermic Patients

ICSI is a successful treatment for severe male subfertility. Only a single viable spermatozoon is needed to fertilize a single oocyte. Even if spermatozoa cannot be found in the ejaculate, surgically retrieved spermatozoa from testes or epididymis are available for ICSI. In fact, a surgical procedure to retrieve sperm from the testes (i.e., TESE) is essential for NOA. Recently, the indication of TESE was extended to patients with severely impaired spermatogenesis, especially cyptozoospermia. It is known that an extremely low sperm count has a negative effect on the outcome of ICSI [7]. It is postulated that

Table 4. The comparison of ICSI cycles with ejaculated sperm and testicular sperm

		Ejaculated sperm	Testicular sperm	
Bendikson [36]	cycles	27	21	
	embryos transferred	3.0 ± 0.8	3.1 ± 0.8	
	pregnancy rate per ET	20.8%	47.4%	
	live birth rate per ET	20.8%	42.1%	
Hauser [37]			Fresh	Frozen
	cycles	34	9	50
	embryos transferred	2.3 ± 1.7	3.0 ± 2.3	2.8 ± 1.4
	pregnancy rate per ET	14.3%	42.9%	12.8%
	live birth rate per ET	14.3%	42.9%	12.8%
Ben-Ami [38]	cycles	68	48	
	embryos transferred	1.8 ± 1.2	2.5 ± 1.5	
	pregnancy rate per ET	15.1%	42.5%	
	live birth rate per ET	9.4%	27.5%	

ET, embryo transfer.

sperm may be susceptible to damage during passage through the male genital tract [33]. Therefore, when fertilization fails or implantation is not achieved, patients with an extremely low sperm count are candidates for TESE.

Weissman *et al.* [34] reported a series of 4 couples with male factor infertility (severe oligoasthenteratozoospermia in all cases) and multiple IVF/ICSI failure with poor embryo quality and repeated implantation failure using motile ejaculatory spermatozoa. The use of fresh testicular spermatozoa cells resulted in pregnancies in all cases. Surprisingly, the patients had 10, 9, 8, and 4 IVF/ICSI failures, respectively, using ejaculate spermatozoa before using testicular spermatozoa.

Bendikson *et al.* [35] compared the outcomes of ICSI cycles using ejaculate spermatozoa with those of cycles using fresh testicular spermatozoa in 16 couples with cryptozoospermia who underwent ICSI using either ejaculate or fresh testicular sperm cells. There was no significant difference in the fertilization rate between the ejaculate sperm and testicular sperm groups (51.7% vs. 59.9%); however, the rate of clinical pregnancies and deliveries showed a trend in favor of the group in which testicular spermatozoa were used (47.4% vs. 20.8% and 42.1% vs. 20.8%; Table 4).

Hauser *et al.* [36] reported a significantly higher fertilization rate following ICSI with fresh or frozen-thawed testicular spermatozoa than in ICSI with ejaculated spermatozoa in 13 couples diagnosed with either cryptozoospermia or virtual azoospermia (the occasional presence of few spermatozoa in the ejaculate). There were no significant differences in the pregnancy rates and live birth rates between the ejaculate spermatozoa and testicular spermatozoa groups (Table 4). However, the use of fresh testicular spermatozoa tended to achieve a good result and yielded significantly better implantation rates than either the frozen testicular spermatozoa or the ejaculate groups (18.5% vs. 5.7% and 5.1%, $P < 0.05$).

Ben-Ami *et al.* [37] compared the outcomes of ICSI cycles using ejaculated spermatozoa with those cycles using fresh testicular spermatozoa in 17 couples with cryptozoospermia, who all underwent TESE after failure of ICSI with ejaculated spermatozoa. Although there were no significant differences in fertilization rates between the two subgroups, ICSI with testicular spermatozoa showed a significantly higher implantation rate (20.7% vs. 5.7%), pregnancy rate (42.5% vs. 15.1%), and delivery rate (27.5% vs. 9.4%; Table 4). Multivariate logistic regression analysis revealed three significant predictors of pregnancy, the use of testicular spermatozoa [OR 5.1, 95% confidence interval (95% CI) 1.8–14.8], use of motile spermatozoa (OR 12.9, 95% CI 2.1–79.1), and female age (OR 0.83, 95% CI 0.7–0.9).

In summary, ICSI with testicular spermatozoa could be a good option for patients with an extremely low sperm count, especially cryptozoospermia or virtual azoospermia, considering the spermatozoa damage that may take place during centrifugation and the uncertainty about the retrieval of good quality spermatozoa on the day of ICSI. Furthermore, severe oligozoospermic patients could be candidates for TESE/ICSI after multiple ICSI failures with ejaculated spermatozoa. In any case, fresh TESE/ICSI is a preferable alternative, considering that the rate of sperm retrieval from the testes is over 90% and that the outcome tends to be better than ICSI with ejaculated spermatozoa.

Genetic Disorder in the Patients with severely Impaired Spermatogenesis

It is known that genetic disorders are more frequently detected in subfertile males than in the general population. Based on the frequency of chromosomal aberrations in patients with different sperm concentrations, karyotype analysis is suggested for men with azoospermia or oligozoospermia with <10 million/ml spermatozoa, and Y chromosome microdeletion screening is suggested for all infertile patients with <5 million/ml spermatozoa [38, 39]. Y chromosome microdeletions are the most important genetic causes of NOA, and the region where microdeletions are detected is named the azoospermia factor (AZF) region. Deletion patterns in the AZF region are mainly classified into AZFa, AZFb, AZFc and AZFb+c [40], and patients with AZFa, AZFb and AZFb+c deletions have no chance of sperm retrieval by TESE. Therefore, NOA patients should be tested for Y chromosome microdeletions before TESE or varicocele repair. Similarly, patients with a sperm count <5 million/ml should be screened for Y chromosome microdeletions before varicocele repair because these patients have no hope of a recovery of sperm count even if varicocele repair is successful. Recently, a novel detection kit for Y chromosome microdeletions in Japanese males has been developed [41] and the examination is now available for clinical use in Japan.

Conclusion

Although there is insufficient evidence to draw conclusion about the effectiveness of varicocele treatment for patients with severely impaired spermatogenesis, including azoospermia and TESE for severe oligozoospermia, these options should be considered as one of the strategies offered to patients. To clarify the outcomes of these strategies, further well-designed research should be conducted. Severe oligozoospermic patients with a clinical varicocele should undergo analysis of karyotype and Y chromosome microdeletions before treatment, because genetic disorders can be negative risk factors for the treatment. In conclusion, these options should be offered to suitable patients to improve the results of reproductive treatment from the aspect of male factor infertility.

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