Running title: TESE in SCI patients

Testicular sperm extraction in patients with spinal cord injury-related anejaculation

Toshiyuki Iwahata,[1] Takeshi Shin,[1,2] Yukihito Shimomura,[1] Keisuke Suzuki,[1] Tomohiro Kobayashi,[1] Akane Miyata,[2] Yoshitomo Kobori,[1] Shigehiro Soh,[1] and Hiroshi Okada[1,2]

[1] Department of Urology, [2] Center for Reproductive Medicine

Dokkyo Medical University Koshigaya Hospital, Saitama, Japan

2-1-50 Minamikoshigaya, Koshigaya, Saitama 343-8555, Japan

Corresponding author: Toshiyuki Iwahata, M.D.

Department of Urology, Dokkyo Medical University Koshigaya Hospital

2-1-50 Minamikoshigaya, Koshigaya, Saitama 343-8555, Japan

Tel.: +81-48-965-8743; Fax: +81-46-965-8743

E-mail: iwahata@dokkyomed.ac.jp

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Abstract

Objectives: To review the results of testicular sperm extraction and intracytoplasmic sperm injection (TESE-ICSI) in patients with ejaculatory dysfunction due to spinal cord injury (SCI) as a method of fathering biological children at a single institution.

Methods: A total of 52 male patients with ejaculatory disorder due to SCI who underwent TESE were included in this study. We investigated the sperm retrieval rates (SRRs) and pregnancy rates (PRs) from the medical records. Data on the age, testicular volume, the hormonal status (lutenizing hormone (LH), follicle stimulating hormone (FSH), testosterone), and the period from SCI injury were also obtained and analyzed to detect any associations with the presence of spermatogenesis.

Results: Of the 52 patients with ejaculatory dysfunction due to SCI, testicular sperm retrieval was possible in 42(80.7%). ICSI was performed in 37 cases, and pregnancy was achieved in 32 cases (pregnancy rate 86.5%). The take-home baby rate (birth rate) was 70.2%. In the group with successful sperm extraction, the testicular volume was significantly greater, the length of time from SCI to extraction was significantly shorter, and serum LH and FSH levels were significantly lower. Serum FSH levels contributed

the most to the feasibility of sperm retrieval by TESE in patients with ejaculatory dysfunction due to SCI.

Conclusions: Results from TESE-ICSI are favorable in patients with ejaculatory dysfunction due to SCI; however, early TESE is recommended because sperm retrieval becomes more difficult as the length of time from SCI increases.

Key Words: anejaculation, infertility, intracytoplasmic sperm injection, spinal cord injury, testicular sperm retrieval

Abbreviations & Acronyms

ART = assisted reproductive technologies

C-TESE = conventional- testicular sperm extraction

EEJ = electroejaculation

FSH = follicle-stimulating hormone

ICSI = intracytoplasmic sperm injection

IUI = intrauterine insemination

LH = luteinizing hormone

micro-TESE = microdissection-testicular sperm extraction

PVS = penile vibratory stimulation

SCI = spinal cord injury

SRR= sperm retrieval rate

T = testosterone

TESE = testicular sperm extraction

Introduction

Spinal cord injury (SCI) is reported to occur often in males of reproductive age, in their 20s to 30s. In male SCI patients, the incidence of erectile dysfunction and ejaculatory dysfunction is high, and even if one could ejaculate, the rate of conception is considered to be only 10% without medical assistance because semen quality may be poor.

For male patients with ejaculatory incompetence due to SCI, ejaculation can be induced by electroejaculation (EEJ) or penile vibratory stimulation (PVS). With sperm retrieved from the collected semen, fathering biological children has been made possible by the intravaginal insemination (home insemination) or by the use of assisted reproductive technologies (ART) such as intrauterine insemination (IUI) and intracytoplasmic sperm injection (ICSI).[1]

Although the quality of ejaculated semen of SCI patients was not favorable, the results for IUI and IVF/ICSI using those sperm collected by ejaculation using various methods were considerably good.[2] Other report identified that ART are not always favorable.[1] On the other hand, it has been reported that the results for ICSI using testicular sperm collected by testicular sperm extraction (TESE) are equivalent to those

reported for obstructive azoospermia.[3]

In Japan, PVS or TESE are performed routinely because EEJ has not covered by the social insurance. Our facility is a university-based tertiary medical institution and we receive referrals of patients who could not achieve ejaculation by PVS at another institution. In this study, we retrospectively investigated male SCI patients affected by ejaculatory dysfunction that underwent TESE and evaluated the sperm retrieval rates (SRRs), pregnancy rates (PRs), and birth rate.

In addition, we evaluated the factors that influence sperm retrieval in these patients.

Methods

Patients

The study included 52 patients with ejaculatory dysfunction due to SCI who wished to father children, who were referred to the Department of Urology at Dokkyo Medical University Koshigaya Hospital from April 2006 to August 2014. These were patients who either could not tolerate sustained adequate stimulation of the penis for

ejaculation due to autonomic hyperreflexia or those who could not ejaculate with PVS.

Data collection

We retrospectively investigated patient background by reviewing medical charts, including age at the time of clinic visit, age at the time of SCI, length of time between SCI and initial evaluation, testicular volume, and data from endocrine exams (follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T)).

In addition, we conducted a telephone survey on whether sperm retrieval was feasible, and in cases where mature sperm could be collected, the outcome of pregnancy/delivery in their partners. Pregnancy rate and birth rate (take-home baby rate) per patient and per treatment cycle were calculated. SRRs were defined as the rate of retrieval of mature, motile spermatozoa that could be used for ICSI. This study was conducted with the approval of Dokkyo Medical University Koshigaya Hospital Ethics Committee/Internal Review Board. (Approval Number Koshigaya 25077)

Operative procedures

As for the TESE procedure, we performed conventional TESE (C-TESE) under general anesthesia, where scrotal skin was incised approximately 5 mm, the tunica

albuginea was incised through this skin opening, and a small amount of testicular tissue was harvested. If mature spermatozoa could not be identified intraoperatively during testicular tissue retrieval, the scrotal skin incision was extended, the testicle was dislodged externally, and using methods described previously, microdissection TESE (micro-TESE) was performed under an operating microscope.[4] Also, if spermatozoa could not be collected with micro-TESE on one side, the same surgical procedure immediately was repeated on the contralateral side.

Statistical Analysis

Mann-Whitney U-test was used to compare age, testicular volume, serum hormone levels (T, FSH, LH), and length of time between SCI and surgery between those with successful sperm extraction and those with failed sperm extraction. Univariate analysis was performed for factors that influence sperm collection using micro TESE, and for those factors that showed significance, multivariate analysis was performed. Effect of period after SCI on spermatogenesis was evaluated by contingency table analysis. SPSS v18.0 (IBM, Armonk, NY) was used for the statistical analyses. Statistical significance was defined as $p<0.05$.

Results

Of the 52 patients with ejaculatory dysfunction due to SCI, testicular spermatozoa could be retrieved in 42 cases (80.7%). ICSI was conducted in 37 cases, and pregnancy was achieved in 32 cases (pregnancy rate per couple and per ICIS cycle was 86.5% and 34.3%, erspectively). All were single pregnancies. ICSI was not implemented in 5 cases because the partner was awaiting treatment for uterine fibroids or under therapy for ovulatory disorders. In the 37 cases where ICSI was performed, 26 cases resulted in a viable infant (take-home baby rate 70.2%).

C-TESE was performed as the method of testicular sperm retrieval in 26 patients whose testicular volume exceeded 10ml. 8 patients of them for which collection could not be achieved with C-TESE were converted to micro-TESE. Eventually all 26 patients succeeded in testicular sperm retrieval.

In 18 patients with a small testicular volume less than 10 ml and a strong suspicion for a high degree of spermatogenic dysfunction, micro-TESE was performed

from the beginning.

The median age at the time of TESE was 36. The median length of time since SCI was 14 years. The median testicular volume was 16 ml. In the endocrine exam, median serum LH, FSH, and T concentrations [referential value] were 5.2 mIU/ml [2.2~8.4], 7.15 mIU/ml [1.8~12.0], and 5.36 mIU/ml [2.00~7.60] respectively.

In order to evaluate the factors influencing spermatozoa collection with TESE, the successful and failed groups were compared with regards to age at the time of TESE; testicular volume; serum LH, FSH, T values; and the length of time from SCI (Table 1).

Results from univariate analysis revealed that in the successful group, testicular volume was significantly larger (16.18 ml vs. 12 ml), the time from SCI was significantly shorter (12.7 years vs. 25 years), and serum LH (5.74 mIU/ml vs. 14.3 mIU/ml) and FSH (7.46 mIU/ml vs. 33.7 mIU/ml) were significantly lower. There were no differences between the two groups with regards to age at the time of TESE and serum T.

Next, in order to analyze the factors contributing to the success of sperm collection, multivariate analysis was performed on testicular volume, time from SCI, serum LH, serum FSH, all of which had showed significant difference between the two groups in the

univariate analysis.

Consequently, we found that serum FSH contributed the most to successful sperm collection with TESE in SCI patients (Table 1). For evaluation of the influence of the period after SCI on spermatogenesis, patients who underwent TESE were divided into two groups. Group S consisted of patients who were injured less than 12 years, and group L consisted of patients who were injured more than 12 years ago. Mean Johnsen Score Count (JSC) with standard deviation of group S (6.48±1.10) was significantly better than that of group L (5.01±2.41) (p=0.049). Sperm retrieval rate (SRR) was evaluated according to the period after SCI. SRR of group S was significantly better than that of group L (100% vs. 67%, p=0.045). (Table 2)

Discussion

It is known that fertility is decreased in male SCI patients because of erectile dysfunction, ejaculatory dysfunction, and abnormalities in semen quality.[5] Infertility treatments for male SCI patients have progressed rapidly in the last 30 years. This is attributed to the development of EEJ and high amplitude vibrators as methods of

collecting sperm from male SCI patients in order to father biological children, as well as the establishment of ICSI.

Although there are reports that EEJ has low side effects and has favorable ejaculation induction rate,[6] there have also been reports in recent years that pregnancy rates are low in ICSI because of low activity in semen collected with EEJ.[7] Additionally, even though PVS has the advantage of sperm collection at home without the need for specialized management at medical facilities, it has the risk of inducing autonomic hyperreflexia, raising concerns about safety, so appropriate training is required on its initiation.[8] On the other hand, there are other reports showing favorable success rates using sperm collected EEJ or PVS.[9-10]

All patients evaluated in this study were patients who could not induce ejaculation with PVS administered at prior medical institutions. In addition, because the use of EEJ is not yet covered by the social insurance in Japan, we consider TESE-ICSI as the standard treatment for patients with SCI-related anejaculation to father biological children at our facility.[11] Also for SCI patients, even if ejaculation could be brought about, a decrease in sperm motility percentage, decrease in sperm survival rate, presence of

white blood cells in semen, and increase in sperm DNA fragmentation index have been identified in the evaluation of semen quality.[12-16] These have contributed to using TESE-ICSI as first choice of treatment as well. We need to notice that Ibrahim and Brackett reported that DNA fragmentation rate of ejaculated sperm was high, they also revealed that the pregnancy rate using these sperm was considerably good.[12]

It is noteworthy that our data suggested spermatogenesis in SCI patients might deteriorated over time, and identified for the first time that the success rate of sperm recovery by TESE dropped after 12 years from SCI. Age of SCI is an important factor influencing spermatogenesis there after. Celigoj et al [17] reported that SCI before the age of 9 years appeared to interfere with spermatogenesis, and semen quality of those injured near the age of 12 years was not impaired. In the present study only one subject was below the age of 18 years when injured. Thus we cannot evaluate the effects of SCI before puberty.

Furthermore, we have evaluated the factors that influence the feasibility of sperm retrieval using TESE in SCI patients with ejaculatory dysfunction. As a result, those with higher testicular volume and lower LH, as well as lower FSH, had higher rates of sperm

collection using micro-TESE. It is thought that this is reflective of how, in general, spermatogenesis is favorable in larger testicular volumes without the elevation of gonadotropins.[18]

In this study, we also looked into the length of time from SCI to surgery and the SRR. We found that there is a trend of lower SRR when the length of time from SCI is longer. Additionally, we found that serum FSH was the most significant factor affecting sperm retrieval in our results from multivariate analysis. It is thought that this is crucial information when obtaining informed consent for performing TESE in SCI patients.

The ICSI results using sperm collected by TESE revealed pregnancy rate of 78% and pregnancy rate per ICSI cycle was 43.4%. Birth rate per couple was proven 63.4%. We found that these rates were just as favorable as TESE-ICSI pregnancy rate of 76% and take-home baby rate of 65% for patients with obstructive azoospermia at our facility.

Based on these current results, for SCI patients of reproductive age where there is ejaculatory incompetence on assessment of sexual function, we have to proactively inform them about early testicular sperm retrieval and cryopreservation, and collect sperm with TESE and cryopreserve them in those who desire the procedure. In addition,

there is a trend of more proactive involvement in rehabilitation in cases of successful sperm cryopreservation (unpublished data). Currently, we are investigating the effects of sperm cryopreservation on the extent of recovery of activities of daily living and psychological aspects resulting from subsequent rehabilitation.

Conflicts of interest

None daclared.

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