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LETTER TO THE EDITOR

Operational Andrology

A new simple technique of epididymal sperm collection for obstructive azoospermia

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Dear Editor,

Sperm in patients with obstructive azoospermia (OA) have been retrieved from the reproductive tract and/or from the testis for intracytoplasmic sperm injection (ICSI) in several ways, including microsurgical epididymal sperm aspiration (MESA),[1] percutaneous epididymal sperm aspiration (PESA),[2] testicular sperm extraction (TESE),[3] and other techniques.[4] Each of these has advantages and drawbacks in terms of microsurgical expertise or equipment, number of sperm obtained, invasiveness, and cost. Therefore, we developed a safe, simple, feasible, and low-cost modified MESA technique to collect epididymal sperm under direct vision, which we have labeled macrosurgical epididymal sperm imprint collection (MESIC). Herein, we present the details of the MESIC technique and our initial experience in patients with suspected OA.

Macrosurgical epididymal sperm imprint collection was performed in 17 patients with suspected OA (mean age: 36 years) from 2002 to 2014. The present study was approved by the Institutional Review Board of Jichi Medical University, and informed consent was obtained from all patients. Evaluations included history taking, a physical examination, and semen analysis including centrifuged pellet analysis on at least two occasions. They had normal ejaculate volume (mean ± standard deviation [s.d.], 3.6 ± 1.0 ml), palpable vasa deferentia, normal-sized testis (mean ± s.d., 15.5 ± 1.7 ml), normal karyotype and normal serum levels of follicle stimulating hormone (median ± s.d., 5.7 ± 2.9 mIU ml^{-1}), luteinizing hormone (mean ± s.d., 3.7 ± 1.6 mIU ml^{-1}), prolactin (mean ± s.d., 7.7 ± 3.7 ng ml^{-1}), and total testosterone (mean ± s.d., 4.6 ± 2.0 ng dl^{-1}). These clinical findings led to the diagnosis of suspected OA.[5,6]

Macrosurgical epididymal sperm imprint collection was performed on the clinically more dilated epididymis under local, spinal, or general anesthesia based on patients' requests. After a small incision of scrotal skin, epididymis was exposed. If needed, scrotal contents were extruded through a small scrotal incision. The epididymal tubules in the proximal portion of the obstructed epididymis were exposed under direct vision (**Figure 1a**). One of the epididymal tubules was cut with a 24-gauge injection needle and/or eye scissors without requiring isolation (**Figure 1b**). Epididymal fluid emerging from the incised epididymal tubule (**Figure 1c**) was harvested simply by touching the base of sterilized disposable plastic dishes (60 mm in diameter), directly to the incised epididymal tubule (**Figure 1d**). The epididymal fluid collected in the plastic dishes was diluted with washing medium and then examined for the presence of sperm directly under an inverted phase-contrast microscope. If the sperm were not detected, MESIC was continued from the corpus to the caput epididymis until motile sperm were obtained. If the sperm were detected, sperm parameters were assessed on a hemocytometer. The retrieved sperm were then frozen in liquid N_2 at −196°C for future ICSI. Diagnostic testicular biopsy was performed in the ipsilateral testis concomitantly with MESIC but was not always necessary. The wound was closed in layers with 4-0 absorbable sutures. All surgical procedures were performed without using a surgical microscope.

Macrosurgical epididymal sperm imprint collection was successfully performed with no intra- or post-operative complications in all 17 patients (100%). The mean ± s.d. of total sperm number ($\times 10^6$), sperm motility (%), and sperm viability (%) were, respectively, 21.2 ± 31.9, 33.0 ± 23.2, and 53.5 ± 25.9. All of the obtained sperm was cryopreserved for future ICSI. Our results that the success rate in obtaining epididymal sperm and the mean percent sperm motility was 100% and 33%, respectively, are comparable to those of previous reports for MESA – success rates of 80%–100% and mean percent sperm motility of 15%–42%[7,8] – suggesting that MESIC is a safe and feasible technique to retrieve epididymal sperm in patients with OA.

Histopathological findings ranged from normal spermatogenesis to defective spermatogenesis with a mean Johnsen score of 8 (range, 4.7–9.0) although epididymal sperm were successfully obtained by MESIC in all patients. These results might be explained by a patchy distribution of reduced spermatogenesis due to chronic obstruction throughout the testis[9] and suggest that histopathological findings based on a single testicular biopsy are not always representative of the entire testis. Mean operative time was 41.5 min (s.d. =7.0) for MESIC alone and 11.7 min (s.d. =4.4) for additional testicular biopsy. Hospitalization period was not short with a mean of 2.8 days (s.d. =0.8). This would be ascribed to some reasons; hospitalization period and anesthesia method were primarily determined by the patients' requests and institutional guidelines: 1-day hospitalization for local anesthesia and 2 or 3 days hospitalization for spinal or general anesthesia. We believe that MESIC could be done on an outpatient basis as is the case for PESA because one patient underwent MESIC under local anesthesia in the present study.

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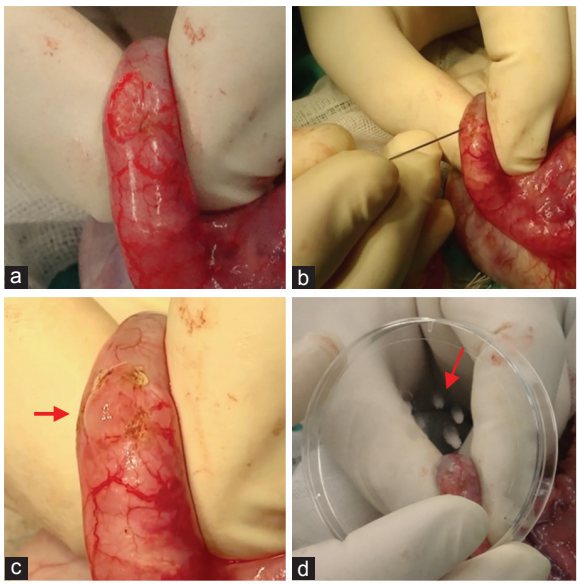

Figure 1: Macrosurgical epididymal sperm collection procedure. (**a**) The epididymal tunic was incised and epididymal tubules in the proximal portion of the obstructed epididymis were exposed. (**b**) One of the epididymal tubules was cut with a 24-gauge injection needle without isolation. (**c**) Epididymal fluid (red arrow) emerging from the incised epididymal tubule was harvested simply by (**d**) touching the base of disposable plastic dishes directly to the incised epididymal tubule.

The advantages of MESIC are as follows. First, a single MESIC procedure, as is the case for MESA, could provide a sufficient number of sperm for cryopreservation for future multiple ICSI cycles, thus avoiding the need for repeated invasive sperm retrieval procedures. Second, MESIC is a safe, simple, and easy technique, which does not require microsurgical skills, under direct vision. Third, MESIC is a low-cost technique without requiring specialized microsurgical equipment. The drawbacks of MESIC includes the followings; it seems to be invasive compared to PESA because the MESIC procedure is very similar to that of MESA, except for the method to collect epididymal fluid; histologic information could not be obtained by MESIC alone although TESE has the advantages of not only sperm retrieval but histologic evaluation. The present study has some limitations, including its retrospective nature, the small number of patients, and lack of ultrasound epididymis assessment and comparison with other techniques for epididymal sperm retrieval. An additional limitation is the lack of subsequent data on fertilization rates and pregnancy rates due to the majority of patients being lost at follow-up or transferred to other hospitals for ICSI as well as cases in which female partners developed malignancy before ICSI.

In conclusion, we developed a new modified MESA technique, MESIC, which is a safe, simple, feasible, and low-cost technique to retrieve epididymal sperm in patients with OA. The choice of method for sperm retrieval from the epididymis in patients with OA depends primarily on the local practice preferences and expertise since the best method to obtain sperm has not yet been identified.[5,10] Thus, the present study suggests that MESIC would be one of the treatment options for patients with OA.

AUTHOR CONTRIBUTIONS

TM designed the surgical technique and drafted the manuscript. TM, MK, ToK, AM, TaK, AF and SK participated in operation and acquisition of data. TM, HK and AT performed data analysis. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

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