Received: 13 October 2017 | Accepted: 24 January 2018

DOI: 10.1002/rmb2.12092

CASE REPORT

WILEY Reproductive Medicine and Biology

Microdissection testicular sperm extraction in five Japanese patients with non-mosaic Klinefelter's syndrome

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Abstract

Cases: Microdissection testicular sperm extraction (micro-TESE) was performed on five Japanese men with non-mosaic Klinefelter's syndrome (KS) and non-obstructive azoospermia in the authors' department. Here is reported the operative results and partner's clinical course for two cases where spermatozoa could be acquired. Also encountered was a man with non-mosaic KS with the partial deletion of azoospermia factor (AZF)b. Because this is rare, it is reported in detail in the context of the previous literature. This case series describes the first experience of micro-TESE by gynecologists in the current department.

Outcome: The egg collection date was adjusted to the micro-TESE day by using the modified ultra-long method. Intracytoplasmic sperm injection (ICSI) was implemented for two men whose spermatozoa were acquired by micro-TESE, with these progressing to the blastocyst stage. Subsequently, one case conceived after the transfer of fresh embryos and a healthy baby was delivered. However, spermatozoa could not be retrieved from the man with non-mosaic KS who was harboring the partial deletion of AZFb.

Conclusion: These findings suggest that ovulation induction by using the modified ultra-long method with micro-TESE and ICSI on the same day represents an effective treatment option for men with non-mosaic KS. As there are cases where AZF deletion is recognized among patients with non-mosaic KS, screening before micro-TESE is strongly recommended.

KEYWORDS

azoospermia, infertility, intracytoplasmic sperm injection, Klinefelter's syndrome, pregnancy

1 | INTRODUCTION

Klinefelter's syndrome (KS) is one of the most frequent chromosomal abnormalities among newborns,[1] and in several cases of KS, non-obstructive azoospermia has been observed.[2,3] Klinefelter's syndrome occurs in ~1 in 500-650 live male births.[1,3-5]

Previously, studies demonstrated that microdissection testicular sperm extraction (micro-TESE) can successfully retrieve sperm in cases of non-mosaic KS with azoospermia.[6-9] Furthermore, there are several reports of healthy pregnancies and the birth of children as a result of such sperm extraction.[6,8] However, there is no clear guidance as to whether the acquisition of a very small number of

sperm from patients with KS should be performed by fresh micro-TESE intracytoplasmic sperm injection (ICSI) or by cryo-micro-TESE ICSI or how the controlled ovarian stimulation (COS) protocol should be selected. Currently, these policies are entrusted to each facility. Furthermore, although many cases of azoospermia factor (AZF) microdeletion in non-obstructive azoospermia have been described,[10] there are few reports of AZF microdeletion in patients with KS.[11,12]

Micro-TESE at Niigata University Medical and Dental Hospital in Niigata, Japan, is led by obstetricians and gynecologists and was first performed in August, 2015. Subsequently, from October, 2015 to April, 2016, micro-TESE was performed on five men with non-mosaic KS whose goal involved having a child. In the two successful cases involving acquired intratesticular sperm, egg collection and ICSI procedures were performed on the same day. By using a modified ultra-long protocol, COS was chosen. One of the two cases established a normal pregnancy, followed by the birth of a healthy baby boy with a normal karyotype (46XY). An examination was performed of the presence or absence of AZF microdeletion in two cases. In both cases, micro-TESE failed to retrieve spermatozoa, and in one of the cases, a partial deletion in the AZFb region (DYS219) was identified. Such a case is very rare.

Here is described the utility of ICSI with fresh testicular sperm that are acquired by micro-TESE in non-mosaic KS, as well as the usefulness of combining the date of egg collection with that of micro-TESE by the modified ultra-long protocol, in the context of previous studies on the subject. Furthermore, it is reported on the need for preoperative AZF microdeletion screening for non-mosaic KS.

In addition to a series of the partner's infertility treatment with assisted reproductive technology (ART), micro-TESE for the men with non-mosaic KS also was performed by the same obstetrician and gynecologist. Support for perioperative and postoperative complications was organized with urologists at the hospital. It also will be explained why obstetricians and gynecologists are required to perform micro-TESE in Japan.

2 | CASE SERIES

The five men with non-mosaic KS that were included in this analysis were all Japanese. Informed written consent was obtained from all the patients for infertility treatment. Micro-TESE for patients with non-mosaic KS was approved by the Clinical Ethical Committee of the hospital and all patients agreed to undergo in vitro fertilization. The background of the five men with non-mosaic KS who underwent micro-TESE are shown in Table 1. All the men were diagnosed with azoospermia by a semen analysis.

The semen samples were obtained from consenting patients after a 2-7 day period of ejaculatory abstinence. The diagnosis of azoospermia was assigned only after the analysis of at least two semen samples per patient. The mean age of the patients with non-mosaic KS was 33.6 years (range: 28-39 years) and an examination of the medical history of all five cases revealed nothing in particular of note. However, Case 4 previously had undergone conventional TESE at another hospital and spermatozoa were not acquired.

TABLE 1 Background of five men with non-mosaic Klinefelter's syndrome who underwent microdissection testicular sperm extraction (TESE) in the authors' department between August, 2015 and April, 2016

Case	Age (y)	Medical history	Volume of testis (cc), right/left (mean)	FSH (mIU/mL)	LH (mIU/mL)	Testosterone (ng/dL)	PRL (ng/mL)	Chromosome (G-band)	AZF deletion status
1	34	No significant finding	2.00/1.60 (1.80)	52.6	18.9	206.20	6.6	47: XXY	Not examined
2	28	No significant finding	2.50/1.40 (1.95)	30.6	12.2	229.10	9.2	47: XXY,inv(9) (p12q13)	Not examined
3	32	No significant finding	3.00/2.70 (2.85)	50.5	14.3	584.40	46.8	47: XXY	Not examined
4	39	Conventional TESE (right testis)	1.00/1.30 (1.15)	60.0	24.5	161.86	14.0	47: XXY	No microdeletion
5	35	No significant finding	3.70/2.00 (2.85)	45.7	16.0	335.00	5.9	47: XXY	AZFb partial deletion
Mean	33.6	–	2.44/1.80 (2.12)	47.9	17.2	303.31	16.5	–	–

AZF, Azoospermia factor; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PRL, prolactin.

TABLE 2 Microdissection testicular sperm extraction (TESE) outcomes

Case	Micro-TESE	Spermatozoa	Operative time	Egg collection day and date micro-TESE was performed
1	Bilateral testis	Positive	1 h, 22 min	Same
2	Unilateral testis	Positive	1 h, 12 min	Same
3	Bilateral testis	Negative	1 h, 38 min	Same
4	Bilateral testis	Negative	1 h, 19 min	Same
5	Bilateral testis	Negative	1 h, 25 min	Same

The testicular volume was measured by ultrasound (testicular volume = length × width × thickness × 0.71). The mean testis volume was 2.12 mL (range: 1.15-2.85 mL). A physical examination was performed to rule out any overlapping anomalies. It was confirmed that there was no cryptorchidism or varicocele by palpation and ultrasound in all cases. The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and prolactin (PRL) levels were measured in blood samples. The mean levels of FSH, LH, testosterone, and PRL were 47.9 mIU/mL (range: 30.6-60.0 mIU/mL), 17.2 mIU/mL (range: 12.2-24.5 mIU/mL), 303.31 ng/dL (range: 161.86–584.40 ng/dL), and 16.5 ng/mL (range: 5.9-46.8 ng/mL), respectively. All cases exhibited the following clinical features: a small testicular size and hypergonadotropic hypogonadism.

A G-banding analysis of the lymphocyte metaphase chromosomes that were obtained from the peripheral blood was performed. At least 20 cells were cytogenetically analyzed per patient. All cases were non-mosaic KS (47, XXY), and in Case 2, inv (9) (p12q13) also was recognized.

To screen for microdeletions in the AZF region of the Y chromosome, a multiplex polymerase chain reaction (PCR) was performed by using the peripheral blood and the Y chromosome deletion detection system.[13] An analysis of the AZF region of the Y chromosome was performed in Cases 4 and 5, with a partial deletion of the AZFb region (DYS219) being observed in Case 5.

After confirming that the patients had consented to treatment, micro-TESE was performed. In all cases, micro-TESE was performed under general anesthesia by the same obstetrician and gynecologist. The micro-TESE outcomes for the five analyzed cases are shown in Table 2. Spermatozoa were retrieved successfully from Cases 1 and 2.

As a characteristic finding from the surgical microscopy of the micro-TESE samples from the five non-mosaic KS cases, stromal tissue that reflected hyperplasia of the Lydig cells was dominant and thin, string-like seminiferous tubules were observed. In Cases 1 and 2, a small number of opaque seminiferous tubules that were thicker than the others were observed and subsequently it was possible to acquire spermatozoa from these tubules. In Cases 3, 4, and 5, there were no thick, opaque seminiferous tubules. Thus, thin seminiferous tubules were extracted carefully and there was an attempt to locate spermatozoa; however, these could not be found.

The surgical microscopy findings for Case 5 as a result of the partial AZFb deletion are shown in Figure 1. In the event that seminiferous tubules were present, they were extremely few. Only fine, thin seminiferous tubules were recognized.

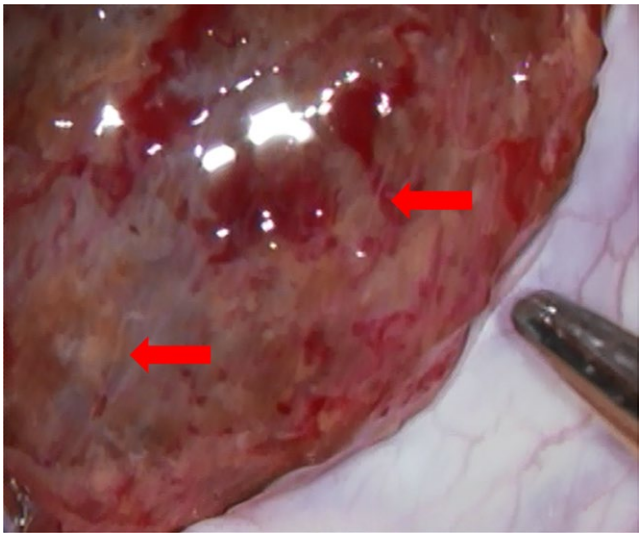

FIGURE 1 Microscopic findings of the testis in Case 5. Expanded seminiferous tubules (magnification: 25×) were unable to be identified and only fine, thin seminiferous tubules were recognized (indicated by an arrow). In the event that the seminiferous tubules were present, they were extremely few

The average duration of surgery was 1 hour and 23 minutes (range: 1 hour, 12 minutes-1 hour, 38 minutes). In the cases where micro-TESE was performed, there were no instances of severe complications, such as acute epididymitis or scrotal swelling. A histological examination of the testis tissue revealed diagnoses of Sertoli-cell-only syndrome in Cases 1, 3, 4, and 5 and maturation arrest in Case 2. The mean Johnsen's score was 1.46 (Table 3).

In particular, the pathological findings for Case 5 with the partial deletion of AZFb are presented in Figure 2. The testis showed extensive tubular hyalinization, nodular Leydig-cell hyperplasia, and fibrosis. A few non-hyalinized tubules contained only Sertoli cells. Although Case 5 had a partial deletion of AZFb, the pathological diagnosis was that of Sertoli-cell-only syndrome.

The testosterone levels were confirmed by an analysis of blood samples at three, six, and 12 months after the micro-TESE. These data are presented in Table 4. In all cases, the postoperative testosterone levels showed a declining trend. Case 1, in particular, reported a tendency toward exhaustion at 12 months after the micro-TESE. Accordingly, Case 1 was referred to the urology department of the hospital for follow-up; however, at 18 months after surgery, the patient's testosterone levels recovered to 95.79 ng/dL without hormone

TABLE 3 Histopathological diagnoses

Case	Pathological diagnosis	Johnsen's score
1	Sertoli-cell-only syndrome	1.0
2	Maturation arrest	3.2
3	Sertoli-cell-only syndrome	1.0
4	Sertoli-cell-only syndrome	1.0
5	Sertoli-cell-only syndrome	1.1

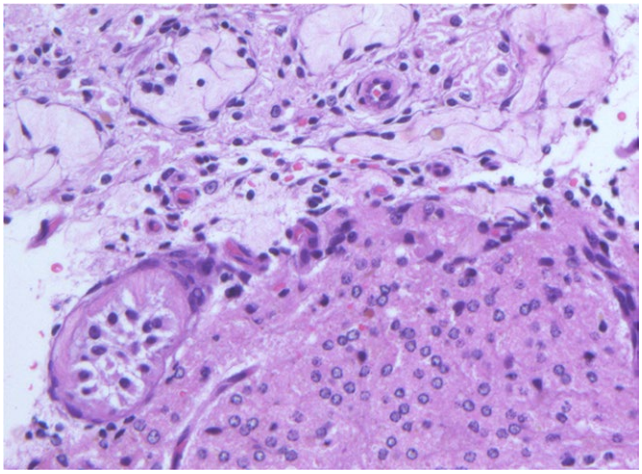

FIGURE 2 Pathological findings in Case 5, with the partial deletion of azoospermia factor b. The testis shows extensive tubular hyalinization, nodular Leydig-cell hyperplasia, and fibrosis. A few non-hyalinized tubules contain only Sertoli cells

replacement therapy. The patient did not hope for androgen replacement therapy. As of September, 2017, none of the five cases required androgen replacement therapy.

The backgrounds and treatment results of the female partners of the examined cases are shown in Table 5. In all cases, the oocyte-retrieval date was adjusted from the wife of the patient to coincide with the day of the micro-TESE. Ovulation induction was performed by using the authors' own method, based on the modified ultra-long protocol.[14] Each patient began treatment with 900 μg/d of gonadotropin-releasing hormone (GnRH) agonist (suprecur) nasal drops from the high-temperature phase of the menstrual cycle and ~30–40 days before the scheduled micro-TESE. After confirming sufficient downregulation, an i.m. injection of 150 IU–300 IU of human menopausal gonadotropin (hMG) was given to the female partner of each case 12 days before the micro-TESE, and the dose of hMG was appropriately adjusted to allow the size of the main follicle of the patient to reach ~18 mm at 2 days before egg collection. Ovulation was individually induced with 5000 IU-10 000 IU of human chorionic gonadotropin. In all cases, more than 5 mature follicles were confirmed by transvaginal ultrasonography, metaphase stage II (MII) eggs were collected, and there was also no onset of severe ovarian hyperstimulation syndrome (OHSS).

Two MII eggs were retrieved from the female partner of Case 1 and ICSI was performed on the same day. The day after the ICSI, one successful fertilization was confirmed and 5 days after egg collection, the fresh blastocyst (3BC by Gardner's classification: full blastocyst, several inner-cell mass counts, very low trophectoderm count)[15] was transplanted; however, the female partner of Case 1 did not conceive. This case is currently receiving ongoing ART using frozen sperm. The partner has not become pregnant to date.

One MII egg was collected from the female partner of Case 2 and ICSI was performed on the same day. The day after ICSI, fertilization was confirmed and 5 days after egg collection, the fresh blastocyst (4AB by Gardner's classification: expanded blastocyst, high inner-cell mass count, low trophectoderm count)[15] was transplanted. Subsequently, a gestational sac was confirmed by transvaginal ultrasound. After egg collection, a natural progesterone preparation (lutinus, vaginal tablet: 300 mg/d) was administered vaginally three times daily as a corpus luteum supplement and conjugated estrogens (Premarin: 1.25 mg/d) were administered for up to 8 weeks of gestation. The pregnancy was uneventful, and at 39 weeks and 2 days of gestation, resulted in the birth of a healthy baby boy weighing 2.866 kg. At 6 months of age, the chromosomes of the child were analyzed and he was found to have a normal 46XY karyotype.

After 18 months from the first childbirth, the partner of Case 2 visited the department, as she wished to conceive her second child. Cryo-micro-TESE ICSI was performed; however, a viable blastocyst did not develop and she has not become pregnant yet.

Cases 3, 4, and 5 requested artificial insemination by using donor semen (AID) after micro-TESE. Accordingly, they were referred to a nearby institution where AID could be performed.

3 | DISCUSSION

Although individuals with KS are generally considered to be sterile, spermatozoa have been observed in the ejaculated seminal fluid in a few cases, and through the use of ICSI, successful pregnancies have

TABLE 4 Preoperative and postoperative testosterone levels (ng/dL)

Case	Preoperation	Postoperation after 3 mo	Postoperation after 6 mo	Postoperation after 12 mo
1	206.20	49.66	62.27	46.55
2	229.10	152.91	174.46	143.87
3	584.40	213.61	243.92	261.99
4	161.86	146.29	94.16	60.91
5	335.00	214.87	212.95	193.52

TABLE 5 Clinical course of the female partner of the patients with non-mosaic Klinefelter's syndrome

Case	Patient age (y)	Method of induction	Total hMG (IU)	hCG (IU)	Mature follicle count (before oocyte pick-up)	Oocyte pick-up (MII)	2PN/2PB	ET	Pregnancy	Course
1	37	Modified ultra-long	1275	10 000	5	2	1	Fresh ET (3BC)	Negative	Continued ART
2	35	Modified ultra-long	1500	10 000	7	1	1	Fresh ET (4AB)	Positive	Baby acquisition
3	33	Modified ultra-long	1050	10 000	7	5	—	—	—	Wish to pursue AID
4	29	Modified ultra-long	1950	10 000	10	4	—	—	—	Wish to pursue AID
5	35	Modified ultra-long	1950	5000	14	14	—	—	—	Wish to pursue AID

2PN/2PB, two pronuclei and two polar bodies (normal fertilized oocyte); AID, artificial insemination using donor semen; ART, assisted reproductive technology; ET, embyo transfer, hCG, human chorionic gonadotropin; hMG, human menopausal gonadotropin, MII, metaphase stage II.

ensued.[16-18] In many cases of KS, spermatogenesis leads to non-obstructive azoospermia[2,3]; however, even in cases of non-mosaic KS that causes azoospermia, sperm can be acquired by micro-TESE. The sperm acquisition rate in KS cases by using micro-TESE has been reported as being from 50% to 65%,[3,9,19,20] which is considered to be relatively high.[20] In the present cases, using micro-TESE, it was possible to acquire spermatozoa in 40% of cases (2/5 cases). The current relatively low retrieval rate is likely related to the small sample size.

It remains unclear whether the supernumerary X chromosome is the underlying cause of testicular failure in KS and the molecular mechanisms leading to testicular failure and poor insemination function in KS have not been fully elucidated.[3] A previous study reported that the frequency of XX and YY disomic and XY hyperhaploid and diploid spermatozoa were significantly elevated in a 47, XXY man, as compared with the participants of proven fertility, and with the second infertile man with a normal constitutional karyotype,[21] with the frequency of gonosomal abnormalities and diploid spermatozoa significantly increased in the KS case.[22] However, there are many reports that the chromosome of an infant that is born from a father with non-mosaic KS had a normal karyotype.[8,23-25] In the present report, the child that was produced from ICSI by using the spermatozoa of Case 2 harvested by micro-TESE had a normal karyotype (46XY). Indeed, spermatogenesis in patients with KS probably occurs similarly to that in patients who have gonadal failure due to other reasons and the sperm are probably the product of a normal germ line.[23] Concerns have been raised about the genetic risk to the offspring of patients with non-mosaic KS; however, this risk has not been found to be greater than that of patients with non-obstructive azoospermia with a normal karyotype.[26] Irrespective of the actual and perceived genetic risks, it is essential that patients and their partner receive adequate and comprehensive genetic counseling before treatment.

In this study, oocytes were collected and the micro-TESE was performed on the same day to avoid damage to the sperm caused by freezing. Comparative studies using fresh or frozen sperm in TESE-ICSI in non-mosaic KS demonstrated that the use of fresh sperm resulted in improved fertilization and clinical pregnancy rates.[27] Furthermore, on dividing patients with azoospermia receiving micro-TESE ICSI into KS and normal-karyotype groups, it was shown that the fertilization rates were consistently higher in the KS patient group (KS group vs. normal karyotype group: 28% vs 21%; $P = .046$). Furthermore, although there was no significant difference, the live-birth rate tended to be higher (KS group vs normal-karyotype group: 13% vs 3%; $P = .05$).[28]

As there was only one opportunity to use fresh testicular spermatozoa, the COS protocol was carefully planned: specifically, the most optimal method for collecting sufficient MII eggs, while avoiding the risk of OHSS. Moreover, the COS protocols with a low risk of being cancelled on the day of the micro-TESE were considered, as well as the GnRH protocol and flexibility in starting the ovarian-stimulation protocol.[29]

Regarding flexibility in starting the ovarian-stimulation protocol, a previous report showed the starting times of ovulation induction

divided into three groups: the conventional ovarian-stimulation group (starting on menstrual cycle days 2-5); the late follicular-phase ovarian-stimulation group (starting at menstrual cycle days 6-14); and the luteal-phase ovarian-stimulation group (starting in the luteal phase after menstrual cycle day 14). The authors reported no difference between the results for the acquired eggs or the clinical pregnancy rate between the three groups; however, the cancellation rates were 10.0%, 22.0%, and 16.0%, respectively, and the ovarian-stimulation duration (days) were 8.9 ± 1.4, 11.4 ± 3.1, and 10.9 ± 3.4, respectively.[30] Depending on the menstrual cycle, there are variations in the cancellation rates and the number of days of administration of gonadotropin. By contrast, although it is a study about the COS protocol in polycystic ovarian syndrome, there is a report that the cancellation rate was 9.33% and the number of gonadotropin administration days up to egg collection was 11.52 ± 2.15 in the modified ultra-long method.[14]

Therefore, the GnRH agonist protocol was selected and it was evaluated that sufficient egg collection was possible if this was performed 12 days before the micro-TESE; hMG administration started after confirming sufficient downregulation. Using this approach, more than 5 mature follicles were obtained for all cases. Furthermore, there was success in the blastocyst transplants with fresh micro-TESE ICSI in the cases where sperm was retrieved. However, a good blastocyst could not be obtained by ART using frozen spermatozoa for Cases 1 and 2.

Spermatozoa were unable to be retrieved in three of five cases and the oocytes that were obtained on the same day from the female partner were discarded. To avoid unnecessary medical procedures and associated costs, it is necessary to be able to predict the success of micro-TESE. Various approaches have been explored to predict micro-TESE success rates; however, no clear predictor of sperm acquisition has been found.[26]

In cases of azoospermia, screening for AZF deletion prior to the micro-TESE is important for predicting the success of sperm collection. Complete deletions of AZFa usually manifest as Sertoli-cell-only syndrome, the main feature of which is azoospermia. Patients with AZFb deletions show spermatogenesis arrest during the spermatocyte stage:[30,31] sperm cannot be retrieved, even by micro-TESE, in cases with deletions within the complete deletion range of AZFa and those of the AZFb or AZFb+c region of the Y chromosome.[31] However, recent reports have recognized the presence of sperm in the ejaculate of cases in which the partial deletion of AZFb had been observed.[32] Moreover, it was reported that men with complete AZFb deletion (the absence of specific sequence-tagged site markers, sY121, sY127, sY134, and sY143) can successfully produce a baby by ICSI and that the AZFb deletion is inherited.[33]

Cases of KS that are complicated by AZF microdeletions also have been reported. In one report, Y chromosome microdeletions spanning the AZFa and AZFb loci were found in four of the 14 azoospermic patients with KS.[11] Another report identified the AZFb+c+d microdeletion in one case of KS out of 111 cases that were analyzed.[12] However, such cases are rare.

For Case 5, a partial deletion of the Y chromosome AZFb region (DYS219) was detected and it was explained to this patient that the probability of sperm retrieval with micro-TESE was extremely low and that it is never advisable to combine the day of egg collection with that of micro-TESE. However, this man and his partner expressed a strong desire to undergo micro-TESE and egg collection on the same day, as they hoped for the possibility of the acquisition of a small number of spermatozoa.

Although AZF-deletion screening was recommended to all cases, Cases 1, 2, and 3 did not wish to undergo this screening, with the reasons being expressed that "The examination fee is too expensive, as it is not covered by insurance" or "Because the cause of azoospermia is known as KS, the frequency of merging AZF microdeletions could be especially low."

However, KS that is complicated by AZF microdeletion also is recognized, as in Case 5. Although the patient was diagnosed as having KS, it is strongly recommended that screening for the AZF microdeletion occur in these patients. Furthermore, insurance policies should be adapted to cover screening for AZF deletion in Japan, as this is an important predictor of spermatozoa acquisition from KS cases.

Finally, in this case series, the same obstetrician and gynecologist performed the micro-TESE, egg collection, embryo transfer, and follow-up of the testosterone levels after the micro-TESE. Furthermore, with regard to Case 2, the parturition course, chromosomal examination of the baby, and growth-curve analysis mainly also were performed by the above obstetrician and gynecologist. Such practices often are not adopted worldwide. When a couple receives counseling from the same doctor, it is possible to secure enough time to build a doctor-patient relationship before surgery. In addition, it is believed that the options, such as AID, fostering, and adoption, might be more smoothly presented to such patients, as in Cases 3, 4, and 5, for whom it was not possible to acquire spermatozoa by micro-TESE.

Additionally, it is more difficult to perform a micro-TESE safely in patients with extremely small testes, such as those with KS, than in azoospermic patients that do not have KS. Furthermore, long-term endocrinologic follow-up is necessary after a micro-TESE, particularly for patients with KS to detect hypogonadism.[34] At 12 months after surgery, Cases 1, 2, 4, and 5 had testosterone levels that were <200 ng/dL. According to the guidelines, total testosterone levels of <2.0 ng/mL are an adaptation criterion for androgen replacement therapy.[35] A higher probability of metabolic syndrome also is associated with lower levels of serum total testosterone levels in relatively healthy, middle-aged Japanese men.[36] Therefore, it is believed that micro-TESE, including postoperative follow-up, should be performed by reproductive medical professional urologists who are familiar with the dissection of male reproductive organs and andrology.

In Japan, there are 649 reproductive health specialists as of April 1, 2017; however, there are only 51 reproductive health professional urinary doctors among them. Additionally, the latter mainly are located in city centers and therefore are unable to treat cases that arise in areas outside of these cities. The authors' institution is located in the countryside of Japan, an area that is lacking reproductive health professional urologists. Therefore, it is often necessary to refer

azoospermic patients to institutions that are located large distances away. Furthermore, there have been a number of cases in which azoospermic patients are unable to meet repeat transportation expenses to visit distant male infertility institutions for treatment by reproductive health professional urologists. Often, such patients abandon their treatment. Male infertility accounts for one-fifth of all instances of couples failing to conceive, and in 39% of these cases, both the man and the woman present with disorders. Furthermore, one of the main causes of male infertility is azoospermia (9%).[37] Therefore, in order to implement azoospermic patient treatment in the current area so that it is available to those who wish to conceive, the authors learnt the micro-TESE technique at a facility that had already implemented micro-TESE and subsequently conducted this procedure on patients with non-obstructive azoospermia in the authors' hospital under the approval of the hospital Clinical Ethics Committee. The authors believe that specialists in male infertility and andrology are necessary in order to enable the effective treatment of patients with azoospermia, even in rural locations.

ACKNOWLEDEGMENTS

We thank the supporting medical staff for performing the embryo culture in the laboratory. We are grateful to E. Shibaoka and A. Igarashi. We also thank Teiichi Motoyama for guidance on the pathological diagnosis of the testes. We also would like to thank Editage (www.editage.com) for English-language editing and publication support.

DISCLOSURES

Conflict of interest: The authors declare no conflict of interest. *Human Rights Statement and Informed Consent*: All the procedures were followed in accordance with the ethical standards of the responsible committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments. Informed consent was obtained from all the patients included in the study. The Clinical Ethical Committee of Niigata University Medical and Dental Hospital, Niigata, Japan, approved this study. *Animal studies:* This article does not contain any study with animal participants that have been performed by any of the authors.

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How to cite this article: Chihara M, Ogi K, Ishiguro T, et al. Microdissection testicular sperm extraction in five Japanese patients with non-mosaic Klinefelter's syndrome. *Reprod Med Biol*. 2018;17:209–216. https://doi.org/10.1002/rmb2.12092