Kaohsiung Journal of Medical Sciences (2018) 34, 103–108

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journal homepage: http://www.kjms-online.com

Original Article

Microdissection testicular sperm extraction (micro-TESE): Predictive value of preoperative hormonal levels and pathology in non-obstructive azoospermia

Alper Eken [a,b,*], Feray Gulec [c]

[a] Department of Urology, Acibadem Adana Hospital, Adana, Turkey
[b] Acibadem University, Vocational School of Health Services, Istanbul, Turkey
[c] Department of Embryology and Histology, Acibadem Adana Hospital, Adana, Turkey

Received 11 April 2017; accepted 28 August 2017
Available online 19 September 2017

KEYWORDS
Intracytoplasmic sperm injection;
Live birth delivery rate;
Non-obstructive azoospermia;
Testicular sperm extraction;
Sperm retrieval rate

Abstract The aim of this study was to evaluate the predictive value of preoperative hormonal levels and pathology, as well as the outcome of microsurgical testicular sperm extraction in patients with non-obstructive azoospermia (NOA), presenting to our clinic for treatment of infertility. The records of 145 men with NOA who underwent microdissection testicular sperm extraction (micro-TESE) between March 2013 and November 2016 were studied. The patient's age, testicular volume, hormonal profile for follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (TT), and testicular pathology were recorded. The sperm retrieval, the clinical pregnancy and live birth delivery rates were noted. Our testicular sperm retrieval rate was 65.5%. There was no statistical difference in age, testicular volume, or hormonal levels in the TESE-positive and negative groups. Hypospermatogenesis was found in testicular histopathology in 57 of 117 patients (48.7%) who underwent testicular biopsy. Sertoli Cell-Only (SCO) syndrome was seen in 20.5%, Germ Cell Maturation Arrest (MA) in 16.3%, and Atrophy-hyalinization in 14.5%. Seven men had Klinefelter's syndrome (KS), four of whom were TESE-positive. There were no adverse effects of the procedure except for infection at the incision site in one patient. Single intracytoplasmic sperm injection (ICSI) cycles were performed in 92 couples leading to 41 clinical pregnancies and 26 live birth deliveries. Micro-TESE is a safe procedure in experienced hands and provides infertile men with NOA an opportunity to father children. However unselected candidates with NOA should be counselled at the outset that only 17.9% will eventually become biological fathers.

Conflict of interest: All authors declare no conflicts of interest.

* Corresponding author. Acibadem Adana Hospital, Urology Department, Cumhuriyet Cad. No: 66, 01130 Seyhan, Adana, Turkey.
E-mail address: alpereken@yahoo.com (A. Eken).

http://dx.doi.org/10.1016/j.kjms.2017.08.010

Introduction

Azoospermia or lack of sperm in the ejaculate is found in approximately 10% of males who are evaluated for infertility [1]. Of these, 60% are due to NOA, a condition associated with impaired production of sperm [2]. Men with NOA, require sperm retrieval along with intra-cytoplasmic sperm injection (ICSI) for fathering children. Though there may be an absence of spermatozoa in their ejaculate, sperm may still be retrieved in men with NOA because of the presence of isolated foci of active spermatogenesis. Schlegel first described micro-TESE in 1998 [3]. It was developed to combine the advantages of a less invasive approach with an open excisional biopsy. Testicular trauma could be minimised by identifying the zones of active spermatogenesis through optical magnification, using the surgical microscope. Success rates with conventional and more limited sperm retrieval procedures have been reported to be 20% with percutaneous testicular biopsies and up to 45% with open testis biopsies [4]. Successful sperm retrieval has been reported in up to 63% of men undergoing micro-TESE [5,6]. Most studies on treatment of NOA with micro-TESE followed by ICSI with testicular sperm have concentrated on sperm retrieval and clinical pregnancy rates as outcome measures. Few studies have reported live birth rates [7—9]. The procedure is very expensive and the optimal outcome for couples seeking infertility treatment is delivery of a viable offspring. Thus, it is important to determine its live birth rate, so that couples can be counselled appropriately. We describe our experience with micro-TESE in men with NOA, presenting to our clinic for treatment of infertility.

Patients and methods

The records of a cohort of 145 men with NOA who underwent micro-TESE at the Urology Department of the Acibadem Adana Hospital, Adana, Turkey between March 2013 and November 2016. Clinical assessment of infertile men in our unit was done as described by Esteves et al. [10]. The following parameters were recorded for each patient: a detailed medical history, physical examination, including the presence or absence of a varicocele, and a hormone profile. Testicular volume using a standard orchidometer was also noted. Hormonal evaluation included serum follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), and TT. These were done 1—2 months prior to the micro-TESE procedure. Azoospermia was confirmed for all men on two different occasions by testing centrifuged ejaculates according to the World Health Organization (WHO) guidelines [11]. G-band Giemsa Karyotype was done when indicated. None of the men received exogenous testosterone or any medication for optimising endogenous testosterone production. Micro-TESE was done concomitantly to ovarian stimulation and oocyte retrieval in the female.

Techniques

All micro-TESE procedures were performed by the same urologist (AE) with expertise in microsurgery. Procedures were performed under spinal anaesthesia (unless otherwise requested), with the patient positioned on the operating table in a supine position. Microdissection was performed using a floor-standing operating microscope (OPMI Vario/S88 System, Karl Zeiss, Jena, Germany) to expose the seminiferous tubules. Areas with dilated tubules were identified, from which multiple sections of testicular tissue were obtained. These specimens were analysed for the presence of sperm after teasing all of the tubules. Any viable sperm was collected and prepared for use in ICSI. A specimen was taken for histological analysis during the same surgical procedure.

Patients were discharged on the day of operation. Prior to discharge, patients were examined for scrotal haematoma; they were recommended to stay on bed rest and use a scrotal ice pack application for the first 48 h, with removal of the scrotal dressing after 24 h and cleaning of the incision area with soap and water. Oral analgesics were advised for 3 days. Patients were advised to engage in gradual ambulation over a period of 3—4 days, but told to avoid sports, lifting heavy weights, or having sexual intercourse 10 days postoperatively. All patients were advised to report any adverse signs and symptoms, including fever, persistent pain, swelling, bleeding, and excessive fluid leak from the wound.

Preparation of sperm for ICSI

The seminiferous tubules were teased with sterile hypodermic needles, with the contents milked using sterile glass pipettes. The supernatant obtained was centrifuged and the pellets were suspended in 0.2 mL of sperm culture. A drop was examined microscopically for the presence of spermatozoa and motility. The remaining suspension was incubated for a minimum of 1 h to facilitate sperm motility.

Ovarian stimulation, oocyte retrieval, and embryo transfer

Recombinant FSH, human menopausal gonadotrophin or urinary FSH, were used for ovarian stimulation in the female partner. When the follicles reached 17—18 mm, recombinant or human chorionic gonadotrophin (hCG) was administrated. Oocyte retrieval was performed under general anaesthesia and guided by transvaginal ultrasound 34—36 h after hCG administration. Follicular fluid, obtained by oocyte aspiration, was examined for the presence of cumulus-corona-oocyte complexes, which were then chemically denuded with 40 IU/mL of hyaluronidase (Sage, Foster City, CA, USA). The isolated oocytes were then classified according to nuclear maturity, and were maintained in culture until sperm microinjection. ICSI was carried out at 41 h after hCG administration. Oocytes were transferred to a fresh dish of equilibrated medium after injection with a single sperm, where they were maintained till transfer to the uterine cavity. This was done on the third to fifth day using a soft catheter, with transabdominal ultrasound guidance.

We transferred one or two embryos, depending to the patients age or depending on previous attempts. All couples

underwent single ICSI cycles. The outcome of the pregnancy leading to delivery of a live birth or miscarriage was noted in each case.

Definitions

Success of micro-TESE (micro-TESE positive) was reported with collection of any number of motile or immotile spermatozoa that allowed sperm injections to be performed.

Testicular histology was classified based on the predominant histopathological pattern: hypospermatogenesis (reduction in the number of normal spermatogenetic cells), germ cell maturation arrest (GCMA, an absence of the later stages of spermatogenesis), SCOS (the absence of germ cells in the seminiferous tubules) and atrophy-hyalinization.

Serum β-hCG levels were measured 15 days after egg retrieval to determine biochemical pregnancy. An ultrasound was done at 6—7 weeks, and a gestational sac with an embryo showing cardiac activity confirmed a clinical pregnancy.

Ethics

The study was approved by the Institutional Review Board (IRB). Waiver of consent was obtained, since the study involved the analysis of existing medical records.

Statistical analysis

Patient demographics and outcomes of sperm injection cycles were analysed descriptively. The Mann—Whitney U Test and the Fischer's exact test were used for comparison of micro-TESE positive and negative groups.

Results

One hundred and forty-five men with NOA underwent micro-TESE at the Urology Department of the Acibadem Adana Hospital, Adana, Turkey between March 2013 and November 2016. Table 1 shows age, testicular volume, and hormonal levels of men with NOA who were involved with the procedure. Ninety-five of 145 of the men were micro-TESE positive, whereas 50 men were micro-TESE negative. As expected, levels of serum FSH and LH were high in all patients. There was no significant difference in age, testicular volume, or hormonal levels in patients who were micro-TESE positive or negative.

Table 2 shows histopathological findings in 117 men. Fifty-seven of 117 men showed hypospermatogenesis on testicular biopsy, 55 (96.5%) were micro-TESE positive. Seven of the 24 SCOS patients, 8 of the 19 GMCA, and 7 of the 17 atrophy-hyalinisation patients were positive for micro-TESE, respectively. The chi-square test showed that higher sperm retrieval rate occurred in patients with hypospermatogenesis compared to those with SCOS, GMCA, and atrophy-hyalinisation. Fresh sperm was used for ICSI and single cycles were done in all couples. Fresh embryos were transferred in 92 of 95 couples in whom the men were micro-TESE positive. Of 92 women in whom embryo transfer was done, 41 became pregnant as demonstrated by a positive β-hCG. The mean age of women who became pregnant was 29.58 ± 5.46 vs. 31.90 ± 5.99 in those who did not, but this was not statistically significant (see Fig. 1).

Fig. 2 shows the schematic overview of men with NOA who underwent micro-TESE for live births. Of 41 women who became pregnant, 26 had a live birth: i.e., 32 babies (6 sets of twins and 20 singletons), while ten had miscarriages, five were lost to follow up, and one is still pregnant. Thus, the live birth rate in this cohort was 17.9%.

Discussion

Before 1995, couples desiring to have children, but in whom the male partner was diagnosed with NOA, were advised to try donor insemination or adoption. Since the introduction of testicular sperm extraction (TESE), couples had the option of having their own biological children with ICSI. TESE-ICSI is now routinely offered for treatment of male infertility due to NOA. From a psychological and financial viewpoint, it is important to counsel couples having infertility treatment about various options available, with the likely outcomes of each.

The sperm retrieval rate by micro-TESE in patients with NOA in our study was 65.5%. Schlegel reported an increase in the ability to find spermatozoa from 45 to 63% with the use of the microdissection technique in men with NOA [6].

Sperm could be retrieved in four of seven of our patients with KS. Okada et al. reported successful sperm retrieval in 26 of 51 non-mosaic KS patients in their study [12]. Schiff and Koga also reported similar results [13,14].

It has been reported that there is no statistically significant difference in testicular volume between NOA patients in whom sperm could be retrieved and NOA patients in whom sperm could not be retrieved [15]. This phenomenon was also observed in our study.

Table 1 Men's age, testicular volume, and hormone levels using the micro-TESE procedure.

	Total (n = 145)	Micro-TESE positive (n = 95)	Micro-TESE negative (n = 50)	*p* Value
Age (years)	33.40 ± 5.73	32.89 ± 4.77	34.38 ± 7.17	0.14
Testicular volume (mL)	10.42 ± 3.76	10.85 ± 3.90	9.62 ± 3.36	0.06
FSH mIU/mL (Normal 2—7 mIU/mL)	19.04 ± 7.64	18.22 ± 7.38	20.62 ± 7.96	0.07
LH IU/mL (Normal 1.8—8.6 IU/L)	9.39 ± 4.15	8.96 ± 3.53	10.2 ± 5.07	0.09
Testosterone ng/dL (Normal 270—1070 ng/dL)	368.02 ± 141.08	381.42 ± 145.33	342.58 ± 130.26	0.11

Table 2 Histopathological findings of 117 patients with non-obstructive azoospermia.

	Micro-TESE positive	Micro-TESE negative	Chi square
Hypospermatogenesis	55/57 (96.5%)	2/57 (3.5%)	$p < 0.05$
SCOS	7/24 (29.1%)	17/24 (70.9%)	
GCMA	8/19 (42.1%)	11/19 (57.9%)	
Atrophy-hyalinization	7/17 (35.3%)	10/17 (64.7%)	
Total	78	39	

The FSH and LH levels were higher in micro-TESE negative men in our study. Similarly, the testosterone levels were also lower in these men. However, this was not statistically significant. Though levels of FSH generally correlate with the predominant pattern of spermatogenesis, they may not predict isolated areas of spermatogenesis within the testis. Ramasamy et al. demonstrated that the relationship between FSH and the presence of spermatogenesis is not straightforward in men with NOA, including men with KS. They concluded that serum FSH level has a poor predictive value for successful micro-TESE [16].

We successfully retrieved sperm in 96.5% of men with hypospermatogenesis based on histopathology, 42% of men with maturation arrest, and 29% of men with SCOS.

Men with SCOS on histopathology were found to have a much lower rate of spermatozoa recovery compared to men with predominantly hypospermatogenesis (HS) and maturation arrest (MA) [17]. Testicular biopsy was done in 117 of our 145 patients. Despite the promising predictive value of biopsy findings, routine use on all patients before sperm retrieval is not recommended. The micro-TESE procedure may be complicated by postoperative pain, infection,

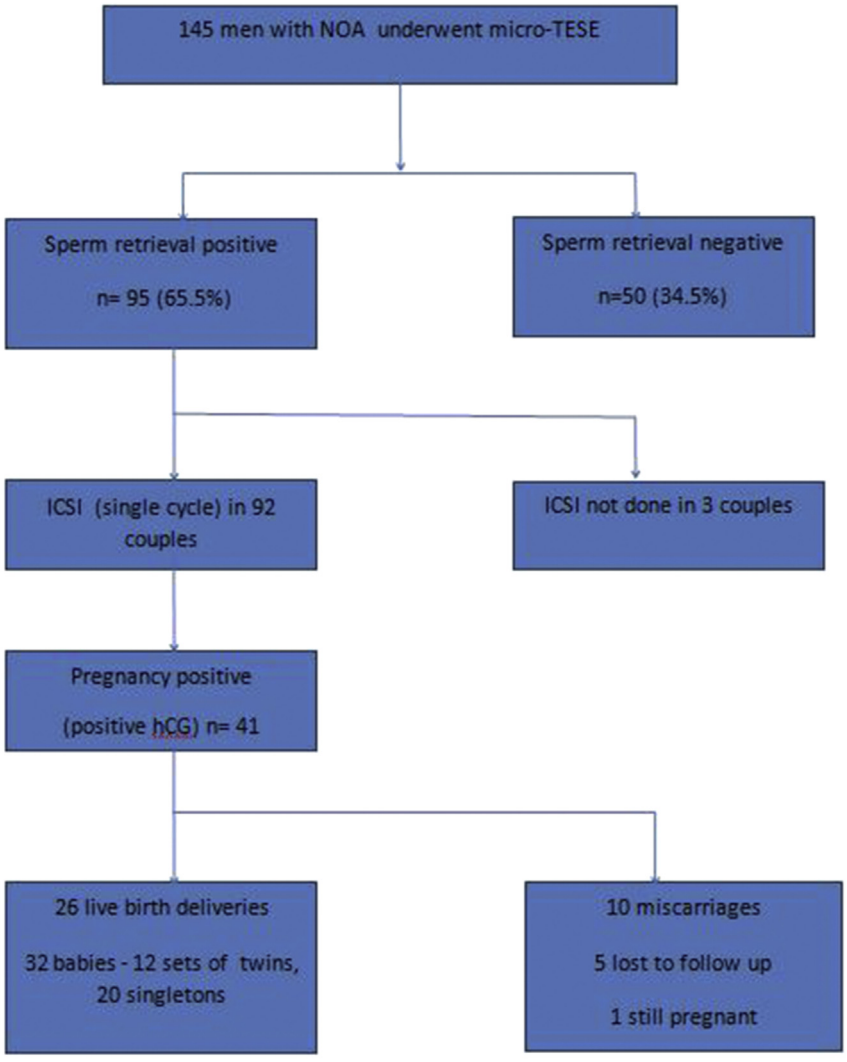

Figure 1. Seminiferous tubules demonstrating hypospermatogenesis with seminiferous epithelial vacuolation.

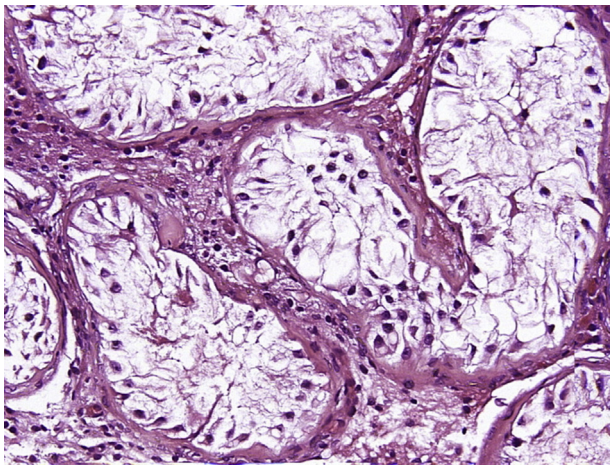

Figure 2. Schematic overview of patients with NOA who underwent micro-TESE until the delivery of live births.

devascularisation, bleeding, and haematoma formation. One patient developed infection at the incision site, but we managed this with oral antibiotics.

EAU Guidelines recommends simultaneous testicular biopsy with micro-TESE to define histopathology and the cause of NOA. We also diagnosed intratubular germ cell neoplasia to see if it existed [18]. According to guidelines, we suggest that patients perform histopathological analysis to define the aetiology of the azoospermia, especially those who had no available prior histopathological analysis. A significant number of patients referred to our clinic had diagnostic testicular biopsy or conventional TESE history in other clinics. Obtained testicle tissue specimen was placed in Bouin's solution and sent for pathological analysis. Twenty-eight patients who had no previous testicle biopsy and histopathological results, vs. patients unwilling to pay since we are a tertiary private fertility centre, in which biopsy or micro-TESE had higher costs, histopathological examination was not done.

The necessity of prior diagnostic biopsy is still controversial in the literature [19—21]. In some clinics, prior diagnostic testicle biopsy was carried out to ensure presence of sperm or for the treatment of patients with NOA [22].

Prior to the micro-TESE, diagnostic testicle biopsy is usually not recommended in the literature due to limited prognostic value; many azoospermic men with NOA will have isolated spermatogenesis foci [23—27]. Prior to micro-TESE, performing a diagnostic biopsy had varied complications, including pain, infection, intratesticular bleeding, or scrotal haematoma, intratesticular scar tissue formation, and decreased androgen production [21]. Moreover, Esteves stresses that prior biopsy may cause removal of the focal spermatogenesis areas, that could jeopardize sperm retrieval rates [24]. Ramasamy and Schlegel emphasise that in finding spermatozoa, there is a need for extensive multiple testicle biopsies; this is because a single biopsy is insufficient and consider that NOA patients cannot be determined prior to diagnostic biopsy to learn who would be candidates for ICSI [27]. Berookhim states that they should stay away from diagnostic testicle biopsy, in that nearly half of NOA patients with negative biopsy results did have sperm in micro-TESE [25]. Abdel Raheem et al. stated that the factor influencing positive sperm retrieval rate is testicular histopathology: they found prior biopsy is not practical, because patients had two surgeries, resulting in increased cost and complication rates [26]. Compatible with the literature, we do not recommend prior open or percutaneous true cut testicle biopsy for azoospermic in practice either.

Single ICSI cycles were performed in 92 of 95 patients in whom sperm was retrieved. These resulted in 41 clinical pregnancies (44.6%) and 26 live births (28.3%). Various authors have reported clinical pregnancy and live birth rates ranging from 26 to 57% for NOA [28—32]. All our patients had single ICSI cycles. Vloeberghs et al. reported a live birth rate of 22% with a single cycle, increasing to 37% with six cycles [7]. In our unselected cohort of 145 men, the live birth rate was 17.9%. This assists men with NOA who present for infertility treatment.

A major limitation of this study is its retrospective study design. In addition, assisted reproduction techniques (IVF or ICSI) as a whole are associated with multiple gestation and elevated risk of congenital abnormalities, compared to the rate of malformations in children conceived naturally [33]. Current data is limited, and hence it is recommended that children conceived by such techniques be followed closely.

Conclusions

Micro-TESE-ICSI is a safe option for treatment of NOA in men who present with infertility. However, couples should be counselled regarding the odds of delivery of a viable offspring.

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