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ORIGINAL RESEARCH

Microdissection testicular sperm extraction in non-obstructive azoospermic patients with solitary testis: a retrospective case-control study

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Abstract

Obtaining sperm from the testis surgically and using these sperm with the intracytoplasmic sperm injection technique, has opened the way for the possibility of biological fathering in men with non-obstructive azoospermia (NOA). We aimed to evaluate our sperm retrieval rate (SRR) by microdissection testicular sperm extraction (micro-TESE) in NOA patients with solitary testis. In this retrospective case-control study, forty-five patients with NOA who had a congenital or acquired solitary testis were included, between September 2003 and January 2022. These patients were randomly matched with patients with NOA who had bilateral testes, using a 1:3 matching ratio. We found that SRR by micro-TESE in patients with solitary testis was similar to NOA patients with bilateral testis (51.1% *vs.* 50.4%). Age, infertility period, ejaculate volume, serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone, history of varicocelectomy, history of orchiopexy, testicular stimulation therapy before micro-TESE, testicular volume, genetic status, TESE side, micro-TESE success, complications and histopathological evaluation results of both groups were evaluated, there was a statistically significant difference in only serum FSH and LH levels. There was no difference between the groups in terms of complications and hormonal effects in the early postoperative period. Micro-TESE in NOA patients with solitary testis has similar sperm retrieval and complication rates as NOA patients with bilateral testis.

Keywords

Azoospermia; Testis; Sperm retrieval

Extracción de espermatozoides testiculares por microdisección en pacientes azoospérmicos no obstructivos con testículo solitario: un estudio retrospectivo de casos y controles

Resumen

Obtener espermatozoides quirúrgicamente del testículo y utilizar estos espermatozoides con la técnica de inyección intracitoplasmática de espermatozoides ha abierto la posibilidad de la paternidad biológica en hombres con azoospermia no obstructiva (ANO). Nuestro objetivo fue evaluar nuestra tasa de recuperación de espermatozoides (TRE) mediante la extracción microscópica de espermatozoides testiculares (micro-TESE) en pacientes con ANO y testículo único. En este estudio de casos y controles retrospectivo, se incluyeron cuarenta y cinco pacientes con ANO que tenían un testículo único congénito o adquirido, entre septiembre de 2003 y enero de 2022. Estos pacientes fueron emparejados al azar con pacientes con ANO que tenían testículos bilaterales, utilizando una proporción de emparejamiento de 1:3. Hemos observado que la TRE mediante micro-TESE en pacientes con testículo único fue similar a la de pacientes con ANO que tenían testículos bilaterales (51.1% *vs.* 50.4%). Se evaluaron la edad, el período de infertilidad, el volumen de eyaculado, los niveles séricos de FSH, LH y testosterona, antecedentes de varicocelectomía, historial de orquiopexia, terapia de estimulación testicular antes de micro-TESE, volumen testicular, estado genético, lado de la TESE, éxito de la micro-TESE, complicaciones y resultados de la evaluación histopatológica en ambos grupos. Solo hubo una diferencia estadísticamente significativa en los niveles séricos de FSH y LH. No hubo diferencia entre los grupos en términos de complicaciones y efectos hormonales en el periodo postoperatorio temprano. Micro-TESE en pacientes con ANO y testículo único presenta tasas de recuperación de espermatozoides y complicaciones similares a las de pacientes con ANO y testículos bilaterales.

Palabras Clave

Azoospermia; Testículos; Recuperación de espermatozoides

1. Introduction

Azoospermia, defined as the absence of sperm in the ejaculate, is diagnosed by microscopic evaluation of centrifuged ejaculate samples [1]. This condition is considered the most severe form of male factor infertility and accounts for approximately 10%–15% of male infertility [2]. Azoospermia can result from a deficiency in spermatogenesis (Non-Obstructive Azoospermia (NOA)) or obstruction within the testis and genital tract (Obstructive Azoospermia) [3]. In NOA, which is more common and more severe than the obstructive form, medical treatments are often insufficient to restore spermatogenesis [3, 4]. Intracytoplasmic sperm injection (ICSI) with surgically obtained sperm through conventional or microdissection testicular sperm extraction (micro-TESE) is the only way to ensure that some of these patients will father their biological child [4].

Although no series have been reported in the literature, a clinically solitary testis may be identified in some patients undergoing evaluation for NOA. The presence of a solitary testis can be attributed to congenital factors, including intraabdominal testis, vanishing testis and testicular agenesis, or to acquired factors resulting from surgical interventions such as torsion, trauma, atrophy and tumor-related procedures [5, 6].

In this study, our objective was to assess the sperm retrieval rate (SRR) through micro-TESE in NOA patients with solitary testis.

2. Methods

2.1 Patients

The medical records of 45 NOA patients with solitary testis who underwent micro-TESE between September 2003 and January 2022 were retrospectively evaluated. A control group of 135 NOA patients with bilateral testis was randomly matched with these 45 patients using a matching ratio of 1:3.

2.2 Clinical evaluation

All The patients underwent a thorough medical history and physical examination. Testicular volumes were measured using a Prader orchidometer. To definitively exclude the presence of intra-abdominal testis in the group of patients with solitary testis who did not undergo orchiectomy, diagnostic laparoscopy was recommended. Semen analysis was performed in our embryology laboratory following the World Health Organization guidelines and repeated at least twice per patient to diagnose azoospermia. Follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone levels were measured to evaluate the endocrine functions of the patients. Karyotype analysis and Y chromosome microdeletion analysis were performed using standard protocols. Genetic counseling was offered before the procedure to all patients with genetic abnormalities.

2.3 Surgical technique

Micro-TESE was performed as an outpatient surgery under sedoanalgesia and local anesthesia (spermatic cord block and scrotal skin infiltration). Antibiotic prophylaxis (1 g intravenous cephazolin (Sefazol, 8699541271004, Mustafa Nevzat Ilac San., Istanbul, Turkey)) was administered to the patients before the procedure. After making the median raphe incision, the testis was accessed by passing through the layers of the scrotum. The testicular parenchyma was exposed by tunica incision and parenchymal microdissection was performed to select more opaque dilated seminiferous tubules with the operating microscope. Selected and removed seminiferous tubule samples were evaluated by an embryologist. The obtained sperms were cryopreserved by the embryologist for use in a future ICSI procedure. For patients with bilateral testes, if sperm cannot be obtained from one testis, microdissection was repeated on the opposite testis. Additionally, a small tissue sample was taken for histopathological evaluation.

2.4 Data interpretation

Patients with solitary testis were accepted as Group I, and patients with bilateral testis, which constituted the control group, were considered as Group II. In cases of solitary testis, the origin of the condition was examined, distinguishing between congenital and acquired cases. In acquired cases, the underlying cause, such as atrophy due to an undescended testicle, tumor, or torsion, was assessed. Age, infertility period, ejaculate volume, serum FSH, LH and testosterone levels, history of varicocelectomy, history of orchiopexy, testicular stimulation therapy before micro-TESE, average testicular volume, genetic status, TESE side (left, right or bilateral), micro-TESE success, complications and histopathological evaluation results were compared between groups. Furthermore, the study groups were subdivided into subgroups based on whether sperm was obtained or not. Subgroup analysis was then conducted using the aforementioned parameters.

2.5 Statistical analysis

Kolmogorov-Smirnov test or Shapiro-Wilk test was used to determine whether the distributions of continuous variables were normal. If the continuous variables were normal, they were presented as mean $\pm$ standard deviation. If not, they were presented as median. Parametric and non-parametric continuous variables were compared using Student's t-test and Mann-Whitney U test, respectively. The study analysed categorical variables between the groups using either the Chi square test or Fisher's Exact test. Statistical significance was considered when the p-value was < 0.05. Statistical analysis was performed using Statistical Package for the Social Science (SPSS) version 26.0 (SPSS Inc., Chicago, IL, USA).

3. Results

In Group I, 32 (71.1%) patients had a history of orchiectomy, while 13 (28.9%) patients had unilateral non-palpable testis. Of the 32 patients with a history of orchiectomy, 29 (90.6%) had orchiectomy for atrophy due to undescended testis, 2 (6.3%) for testicular tumor, and 1 (3.1%) for testicular torsion. None of the 13 patients with solitary testis who did not undergo orchiectomy accepted diagnostic laparoscopy. Three patients with unilateral non-palpable testis had a history of inguinal exploration in addition to imaging methods to investigate the non-palpable testis. Patient characteristics and statistical analysis results are presented in Table 1.

All preoperative parameters of both groups were evaluated. The only statistically significant difference was found in serum FSH and LH levels. In Group I, suitable spermatozoa for the ICSI procedure were obtained in 23 out of 45 cases (51.1%), while in Group II, suitable spermatozoa were found in 68 out of 135 cases (50.4%). There was no statistically significant difference between the two groups in terms of SRR ($p = 1.000$).

In the subgroup analysis based on sperm retrieval, testicular volume was significantly higher in those in whom sperm was found in both groups. Additionally, in Group II, SRR was higher in patients with a history of orchiopexy. However, we were unable to identify any factors that predicted success in sperm retrieval.

Out of the 180 patients, 13 (7.2%) had non-mosaic Klinefelter syndrome. Among these patients, sperm were obtained in only 3 (23.0%) cases. Although the number of patients with Klinefelter syndrome was higher in Group II, there was no statistically significant difference between the groups. However, in Group II, patients with Klinefelter syndrome had a statistically significantly lower rate of sperm retrieval.

None of the patients experienced serious perioperative complications. However, one patient in Group I developed a subcutaneous hematoma and another patient experienced wound dehiscence in the postoperative period. The hematoma was treated under office conditions, while the wound dehiscence resolved with secondary healing. In Group II, three patients experienced wound dehiscence. None of the patients in either group reported testicular volume loss during the early postoperative evaluation. The overall complication rate was 2.7% and there was no statistically significant difference between the groups.

When comparing the histopathological findings, there was a statistically significant difference between the groups, but no difference was found between the subgroups. The dominant pattern was maturation arrest in groups (55.6% in Group I and 91.1% in Group II) and subgroups (56.5% of sperm positive patients and 54.5% of sperm negative patients in Group I, 92.6% of sperm positive patients and 89.6% of sperm negative patients in Group II).

There was no statistically significant difference in endocrine results between the patients' preoperative and 3-month postoperative results in both groups and subgroups (Table 2).

4. Discussion

Retrieving sperm from the testis through TESE and using the obtained sperm with the ICSI method offers NOA patients the prospect of attaining biological fatherhood [7]. Today, TESE and ICSI have become the standard procedure in the treatment of male infertility due to NOA [8]. Congenital or acquired solitary testis can be detected by the basic evaluation of NOA patients, which includes history and physical examination. Although there is no literature support on this subject, we think that the management of these patients is difficult, as invasive procedures to obtain sperm from the testis, such as testicular sperm extraction, may cause increased anxiety for both the patient, the patient's spouse and the surgeon due to possible complications that may lead to unwanted results. In our current study, to our knowledge which is the only series in the literature, we evaluated NOA patients with solitary testis.

TABLE 1. Characteristics and clinical factors of cases and controls.

Clinical factor	Group I				Group II				*p* value
	Total	Sperm (+)	Sperm (−)	*p* value	Total	Sperm (+)	Sperm (−)	*p* value	
n (%)	45 (100.0)	23 (51.1)	22 (48.9)	-	135 (100.0)	68 (50.4)	67 (49.6)	-	
Age (yr)[a]	34.7 ± 5.2	34.5 ± 6.5	35.0 ± 4.9	0.753	33.4 ± 5.6	34.2 ± 5.5	32.6 ± 4.8	0.075	0.159
Infertility duration (yr)[b]	5 (1–19)	4 (1–16)	5 (1–19)	0.708	3 (0.5–22)	3 (1–22)	3 (0.5–16)	0.068	0.187
Varicocelectomy (n)	3	3	0	0.233	37	16	21	0.339	0.003
Orchiopexy (n)	20	7	13	0.075	10	9	1	0.017	0.0001
Medical therapy (n)	6	2	4	0.414	43	23	20	0.713	0.053
Testicular volume (mL)[b]	14 (1–25)	17 (1–25)	5 (3–20)	0.008	10 (1–25)	14 (2–25)	10 (1–20)	0.006	0.396
Ejaculate volume (mL)[b]	3.0 (0.0–5.5)	2.0 (0.1–2.9)	3.5 (0.5–5.5)	0.127	2.7 (0.1–5.8)	2.4 (0.1–5.8)	2.8 (0.5–5.5)	0.211	0.501
FSH (IU/mL)[b]	26.6 (4.4–80.2)	26.1 (4.4–54.7)	27.3 (4.5–80.2)	0.564	18.2 (2.3–65.9)	15.6 (2.3–65.9)	19.8 (2.5–49.4)	0.168	0.021
LH (IU/mL)[b]	9.6 (2.0–30.6)	11.0 (2.0–24.0)	8.9 (2.3–30.6)	0.928	7.6 (0.1–25.7)	7.2 (0.1–25.7)	8.1 (1.6–25.2)	0.402	0.049
Testosterone (ng/mL)[b]	3.7 (1.2–8.4)	3.6 (2.2–8.4)	3.8 (1.2–6.8)	0.340	3.8 (1.4–14.7)	3.8 (1.5–12.1)	3.8 (1.4–14.7)	0.996	0.854
Klinefelter syndrome (n)	1	1	0	1.000	12	2	10	0.017	0.190
Side (n)									
Left	23	12	11		9	9	0		
Right	22	11	11	1.000	38	38	0	0.0001	0.0001
Bilateral	0	0	0		88	21	67		
Complications	2	1	1	1.000	3	2	1	1.000	1.000
Histopathology (n)									
Normal	3	3	0		0	0	0		
Hypospermatogenesis	1	1	0		1	0	1		
Maturation arrest	26	13	13	0.043	123	63	60	0.335	0.0001
SCO	8	1	7		1	0	1		
Dysgenetic hyalinization	4	2	2		8	3	5		
None	3	3	0		2	2	0		
Post-op FSH (IU/mL)[b]	25.0 (4.4–78.0)	25.0 (4.4–65.6)	24.5 (4.8–78.0)	0.882	17.5 (2.6–62.3)	16.2 (2.6–62.3)	18.5 (3.5–47.2)	0.153	0.040
Post-op LH (IU/mL)[b]	10.2 (2.2–31.9)	10.3 (2.2–31.9)	9.7 (2.8–25.9)	0.836	8.0 (1.8–27.2)	7.3 (1.8–27.2)	8.5 (1.8–24.6)	0.372	0.068
Post-op testosterone (ng/mL)[b]	3.4 (1.2–7.9)	3.2 (2.0–7.9)	3.8 (1.2–6.8)	0.763	3.5 (0.8–12.5)	3.7 (1.7–11.6)	3.4 (0.8–12.5)	0.372	0.780

[a], data presented as mean ± standart deviation; [b], data presented as median (minimum-maximum); n, number; yr, year; mL, milliliter; FSH, follicle stimulating hormone; LH, luteinizing hormone; IU, international units; ng, nanogram; SCO, Sertoli cell only; post-op, postoperative.

TABLE 2. Comparison of changes in endocrine functions between groups and subgroups.

	Group I			Group II		
	Total	Sperm (+)	Sperm (−)	Total	Sperm (+)	Sperm (−)
Baseline *vs.* post-op (*p* value)						
FSH	0.993	0.811	0.853	0.736	0.820	0.692
LH	0.657	0.649	0.876	0.519	0.673	0.567
Testosterone	0.670	0.542	0.960	0.470	0.922	0.349

Post-op, post operative; FSH, follicle stimulating hormone; LH, luteinizing hormone.

In a recent meta-analysis of 117 studies, the overall SRR with the TESE procedure in patients with NOA was 47% [9]. While the overall SRR was 50.6% in our study, this rate was 51.1% in the patient group with solitary testis and 50.4% in the control group of the study. There was no statistical difference between the SRR of the patient group with solitary testis and the control group, and this rate also appears to be consistent with the SRR of NOA patients operated on conventional and/or micro TESE.

Clinical and hormonal factors are controversial in predicting the success of TESE in NOA patients [10]. Although many clinical and hormonal factors such as age, testicular volume, serum FSH, LH and testosterone levels have been investigated in predicting the success of TESE in NOA patients, reliable clinical and hormonal predictors for sperm retrieval success have not been defined [11]. In our study, the history of orchiopexy was significantly higher in the patient group with solitary testis and the history of varicocelectomy in the group of patients with bilateral testis. Serum FSH and LH values were statistically significantly different between the study group and the control group. In addition, testicular volumes were significantly higher in patients from whom sperm were obtained in both patient groups. However, consistent with some recent studies reported previously, we also did not find any clinical and hormonal factors to predict sperm retrieval success [11]. Already, we see that the literature has recently pointed to more sophisticated investigations such as several molecular markers in the blood and seminal fluid [10–12].

The prevalence of Klinefelter syndrome, the most common chromosomal disorder in males and seen in approximately 1 out of every 650 newborn males, rises to 3–4% in infertile males and exceeds 10% in azoospermic patients [13, 14]. In our study and its groups, this rate is lower than what is reported in the literature. Although the number of patients is small and there is only one patient in the patient group with solitary testis (total 13 patients), our SRR in this patient group is within the range reported in the literature [15].

Regardless of the method employed, the testicular sperm extraction procedure may carry potential complications, encompassing persistent pain, swelling, infection, bleeding, hematoma, hematocele, hydrocele, testicular damage and a decrease in testosterone levels [16, 17]. The short-term postoperative complication rate linked to micro-TESE in patients with NOA is between 0–10% [18]. The complication rates observed in our patient group with a solitary testis and the control group align with existing literature. Nevertheless, it is crucial to bear in mind that, albeit rare, certain complications may lead to absolute sterility in azoospermic patients with a solitary testis. In the event of such occurrences, there is a potential for medicolegal implications. Therefore, it is extremely important to perform this procedure under conditions of absolute asepsis/antisepsis, avoiding vertical tunical incision and brutal dissection of the testicular parenchyma, and not under/exaggerated bleeding control. Furthermore, antibiotic prophylaxis in azoospermic patients with solitary testis seems reasonable, as debate continues regarding the routine use of prophylactic antibiotics in surgical sperm retrieval [19].

Despite being introduced almost 30 years ago, the effect of TESE on endocrine functions remains controversial. Regardless of the TESE technique, patients may experience a decrease in testosterone levels, but it is widely accepted that baseline testosterone levels improve in long-term follow-up [20]. The early results of our study indicate that micro-TESE does not have negative effect on the endocrine functions of NOA patients with solitary testis.

The main limitations of our study are its retrospective nature and the small number of patients. However, planning a prospective study is difficult due to the rarity of patients with solitary testis. Additionally, we cannot comment on the effect of micro-TESE on endocrine functions due to the lack of long-term endocrine results of the patients. A limitation of this study is that it is not possible to definitively exclude the presence of intra-abdominal testis in patients with solitary testis who did not undergo orchiectomy. This limitation hinders the evaluation of the possible effect on endocrine functions.

5. Conclusions

In this study, we evaluated NOA patients with solitary testis and found that the rate of sperm retrieval by micro-TESE in patients with NOA was comparable to the overall SRR. However, we did not identify any clinical or hormonal factors that could predict the success of sperm retrieval. Furthermore, we concluded that the micro-TESE procedure was safe in this patient group and did not increase the risks during the perioperative and early postoperative period.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

AUTHOR CONTRIBUTIONS

CO and MRG—Conceptualization; Writing-review & editing. CO and MVK—Data curation. CO, MRG and CS—Formal analysis; Methodology. CO—Funding acquisition; Investigation; Project administration. CO, MRG, EH, EC and MVK—Writing-original draft.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Informed consent forms were obtained from all patients after they were instructed about the micro-TESE procedure and its possible complications. The study protocol was approved by our institutional review board (KA24/105).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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