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Research Article

Repeat Microdissection Testicular Sperm Extraction in Azoospermic Men with Nonmosaic Klinefelter Syndrome

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Introduction. To investigate the predictive factors for successful repeat microdissection testicular sperm extraction attempts in patients with Klinefelter syndrome. *Methods*. A total of 28 azoospermic men with nonmosaic Klinefelter syndrome who have received microdissection testicular sperm extraction twice with successful initial microdissection testicular sperm extraction attempts in our institute were studied. Outcome variables (age, serum follicle-stimulating hormone, luteinizing hormone, testosterone, prolactin, and estradiol) of azoospermic men with nonmosaic Klinefelter syndrome and a successful 2nd surgical sperm retrieval attempt (group A) were compared to those with an unsuccessful 2nd sperm retrieval attempt (group B). *Results*. Twenty-one patients (75%) had successful sperm recovery at the 2nd microdissection testicular sperm extraction attempt. The mean testosterone level at baseline and before the 1st microdissection testicular sperm extraction attempt was higher in group A than in group B (2.7 vs. 0.9 ng/mL, $p < 0.01$, and 3.9 vs. 1.1 ng/mL, $p = 0.02$). Receiver operating characteristic curve analysis identified the threshold baseline testosterone concentration (1.5 ng/mL) of patients with Klinefelter syndrome in predicting successful 2nd sperm retrieval attempts and revealed positive and negative predictive values of 94.44% and 60%, respectively. *Conclusion*. Azoospermic men with Klinefelter syndrome presenting with low testosterone levels and successful sperm recovery during the first microdissection testicular sperm extraction procedure are unlikely to retrieve sperm on the 2nd microdissection testicular sperm extraction attempt. Hence, these patients should be properly counseled before sperm retrieval.

1. Introduction

Klinefelter syndrome (KS), which is the most common sex-chromosome aneuploidy encountered in men being evaluated for infertility, accounts for 10% of nonobstructive azoospermic (NOA) cases and affects approximately one in every 600 males [1, 2]. Although azoospermia is typically the major clinical phenotype in men with nonmosaic KS due to extensive seminiferous tubule hyalinization and spermatogonial stem cell depletion, the sperm can be successfully recovered in nearly half of the cases using microdissection testicular sperm extraction (mTESE) due to the presence of preserved intratesticular spermatogenesis foci [3, 4].

With the advent of intracytoplasmic sperm injection (ICSI) using testicular sperm retrieved by mTESE, men harboring this widespread gonosomal aberration are no longer considered sterile, and the probability of paternity is achieved in approximately 10% of KS cases as described in the contemporary literature [4–8]. Along with a considerable body of emerging evidence implicating that pregnancy

outcomes in patients with NOA using cryothawed sperm for ICSI was comparable to when using freshly retrieved testicular sperm [4], using cryopreserved sperm was adopted by some in vitro fertilization (IVF) laboratories to eliminate the need for concurrent oocyte and sperm retrieval and avoid possible unnecessary ovarian stimulation due to failure of surgical sperm retrieval. However, previous adequately powered studies have reported conflicting results regarding the reproductive outcomes of fresh and cryothawed sperm in a specific subset among NOA cohorts, the KS men [9–11]. One of the studies specifically demonstrated that fresh sperm yielded superior outcomes in terms of fertilization and pregnancy rates [9].

The ultimate decision of using whether fresh or frozen-thawed sperm is still dependent on the preference of the IVF program. However, it should be noted that failure to recover viable sperm after thawing accounts for 6-30% of cryopreserved testicular samples from men with NOA [12]. Meanwhile, despite the viability of freshly harvested sperm throughout the ICSI procedure, this fresh-ICSI cycle approach carries the risk of pointless ovarian stimulation in nearly half of female partners due to unsuccessful mTESE if using backup donor sperm is not considered [13].

Generally, multiple ICSI cycles are required to achieve pregnancy in these patients; hence, repeat mTESE for fresh ICSI cycles is necessitated in men with substantial KS [14]. To the best of our knowledge, only one previous study has described their sperm retrieval results on repeat mTESE; however, this study included men with various NOA etiologies. They reported an 82% success rate on the 2nd attempt but clearly demonstrated that no reliable clinically identifiable factor measured before the first mTESE attempt can predict the success rate on repeat mTESE from the multivariate analysis [15]. Since a freshly ICSI cycle might be preferable to achieve an optimal reproductive outcome for men with KS and some will have an unsuccessful repeat mTESE, we aimed to evaluate preoperative clinical determinants associated with successful sperm retrieval with mTESE in these patients.

2. Material and Methods

2.1. Patients. After obtaining approval from the Institutional Review Board (IRB) of Taipei Veterans General Hospital (TPEVGH-IRB 2020-06-016 AC), we examined the medical records of 862 patients who underwent mTESE between 2009 and 2020. Among which, azoospermic patients with nonmosaic KS were retrospectively reviewed. The diagnosis of azoospermia was confirmed at least two consecutive seminal analyses according to the World Health Organization (WHO) criteria [16]. The diagnosis of nonmosaic KS (47, XXY) was confirmed by conventional karyotype analysis according to the standard GTG banding protocol using peripheral blood lymphocyte cultures. Baseline assessment included medical history, a complete physical examination, routine laboratory tests, and testicular size measurement. An endocrinological profile of serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, prolactin, and estradiol (E2) at baseline and before the 1st mTESE attempts was also assessed. The study's results will be presented in adherence with the CONSORT statement.

2.2. Microdissection Testicular Sperm Extraction (mTESE). A small midscrotal incision (approximately 3 cm) was made to expose the testis, followed by an incision on the tunica albuginea to expose the seminiferous tubules. Visualization and excision of large or opaque seminiferous tubules were performed microscopically at 20x~24x magnification as previously described [17]. These excised seminiferous tubules were then examined in a real-time manner by an experienced embryologist for the presence of viable spermatozoa. This was terminated when spermatozoa of sufficient quality and quantity were obtained for intracytoplasmic sperm injection (ICSI). If no sperm was identified on one side of the testis, the procedure was continued on the contralateral side.

2.3. Hormone Optimization Treatment before mTESE. KS with hypogonadism, which is defined as a total testosterone <300 ng/dL, is treated with clomiphene, aromatase inhibitor, or human chorionic gonadotropin (hCG) to increase the endogenous testosterone level. The initial treatment with clomiphene citrate 50 mg was considered for patients with LH within the normal range (1.24-7.8 IU/L), whereas administration of aromatase inhibitors was typically used for men with hypogonadal KS and a testosterone/E2 ratio <10. If an endocrine evaluation followed up at 3 weeks showed that the eugonadal status cannot be restored by either option, twice-weekly hCG injections were administered for at least 8 weeks before mTESE.

2.4. Statistical Analysis. Statistical analyses were performed using the Mann–Whitney *U* test to compare age, testis size, and endocrine profile in men with nonmosaic KS and a successful 2nd surgical sperm retrieval attempt (group A) to those men with KS and unsuccessful 2nd sperm retrieval attempt (group B). Multiple logistic regression analyses were performed to identify factors that were independently associated with successful repeat sperm retrieval in men with KS. To compare qualitative variables such as patient age, testicular volume, endocrine profile, and sperm retrieval rate between patients with KS and patients with NOA or spermatogenic failure due to other contributing etiological factors, the Mann–Whitney *U* test and the Chi-squared test were used to assess the statistical significance. All tests were considered statistically significant at $p < 0.05$.

3. Results

3.1. Cohort Characteristics and Predictive Factors of mTESE Outcomes. A total of 90 azoospermic men with nonmosaic KS underwent mTESE at our institution over the study period. Of the 38 (42.2%) patients with successful initial mTESE attempts, 28 underwent repeated mTESE. Among them, 21 (75%) patients had successful sperm recovery at their 2nd mTESE attempt. Testosterone levels at baseline (initial evaluation before medical treatment) were higher in patients with the successful 2nd mTESE attempt (group A) as compared to patients without sperm retrieval at the repeat mTESE (group B) (mean ± SD: 2.7 ± 1.8 vs. 0.9 ± 0.4 ng/mL,

$p < 0.01$). Testosterone levels before the 1st mTESE attempt for patients with or without medical treatment was also indicative of successful sperm retrieval at the 2nd mTESE attempt (mean ± SD: 3.9 ± 3.9 vs. 1.1 ± 0.7 ng/mL, $p = 0.02$). However, other variables, such as age and other hormone parameters (FSH, LH, prolactin, and E2), were not predictive of a successful repeated sperm retrieval (Table 1). The comparison of patients' clinical characteristics and demographic data is summarized in Table 1. Multiple logistic regression analyses further identified that patients with baseline testosterone level ≥1.5 ng/mL have a greater chance of successful sperm retrieval as compared to patients with baseline testosterone level <1.5 ng/mL ($p = 0.017$) (Table 2). Pathology results from those with unsuccessful 2nd mTESE attempt all presented with a Sertoli-cell-only pattern (7/7, 100%). Follow-up testosterone levels were available for 21 KS men after the successful initial mTESE attempts. Among those with testosterone levels ≥1.5 ng/mL after the first sperm retrieval, the chance of successful 2nd sperm retrieval was 91.67% (11/12), while for those with the testosterone levels <1.5 ng/mL after the first sperm retrieval, the chance of successful 2nd sperm retrieval was significantly lower at 33.3% (3/9), with a p value of 0.005. The average decline of testosterone levels from baseline to the 2nd sperm retrieval attempt in these 21 KS men was 20.3%. However, we found that the percentage of testosterone decline was not associated with the success of the repeat mTESE (19.0% ± 6.5% vs. 20.9% ± 18.3%, $p = 0.90$).

3.2. Receiver Operating Characteristic Curve of Testosterone versus Successful Sperm Retrieval. The optimal baseline testosterone cut-off point (value: 1.5) was attained by receiver operating characteristic (ROC) analysis to maximize detection accuracy (sensitivity and specificity). Using ≥1.5 ng/mL as the cut-off point, 94.44% (17/18) of patients had a successful 2nd sperm retrieval attempt, whereas the sperm retrieval rate on the 2nd attempt was successful in only 60% (6/10) of patients with baseline levels <1.5 ng/mL.

The corresponding threshold value of testosterone as determined by ROC was 90.40%, with a sensitivity and specificity of 80.95% and 85.71%, respectively (Figure 1).

3.3. Comparison of Repetitive Sperm Retrieval Rate in Azoospermic Men with KS and Men with NOA but without Gonosomal Abnormality. Upon comparing the repetitive sperm recovery rate achieved in other 128 patients with idiopathic NOA or spermatogenic failure due to other contributing etiological factors at our institution during the study period, men with KS undergoing repeated mTESE presented with significantly lower sperm retrieval rate on their 2nd attempt (75% vs. 89.1%, $p = 0.048$) (Table 3). Testis size, baseline testosterone, prolactin, and testosterone levels before the 1st mTESE attempt were lower in men with KS; meanwhile, other reproductive endocrine parameters, such as baseline FSH and LH levels, were higher.

Table 1: Potential factors predicting successful sperm retrieval at the 2nd mTESE attempt.

	Group A (sperm +)	Group B (sperm -)	p value
Number	21	7	—
Age	35.3 ± 5.0	36.6 ± 4.2	0.51
Testis size	3.8 ± 2.5	3.1 ± 0.6	0.6
Medical treatment	57.1% (12/21)	85.7 (6/7)	0.17
Endocrine (baseline)			
FSH (mIU/mL)	31.0 ± 13.7	25.3 ± 7.2	0.25
LH (mIU/mL)	17.9 ± 9.7	19.5 ± 8.4	0.51
Test (ng/mL)	2.7 ± 1.8	0.9 ± 0.4	**0.001**
Prolactin (ng/mL)	9.0 ± 3.5	9.4 ± 3.8	0.42
E2 (pg/mL)	21.4 ± 8.9	15.3 ± 5.0	0.14
Endocrine (before 1st mTESE)			
FSH (mIU/mL)	33.1 ± 14.8	22.0 ± 6.9	0.699
LH (mIU/mL)	16.5 ± 7.8	—	—
Test (ng/mL)	3.9 ± 3.9	1.1 ± 0.7	0.02
Prolactin (ng/mL)	10.4 ± 4.1	—	—
E2 (pg/mL)	26.2 ± 15.2	17.5 ± 4.6	0.14

4. Discussions

In the present study, 21 of 28 (75%) men with nonmosaic KS and sperm retrieval on their 1st mTESE attempt also had successful sperm retrieval on their 2nd mTESE attempt. Of all the variables analyzed, the testosterone level at baseline and postmedical treatment were the only predictive factors identified in the study population for successful repeated surgical sperm retrieval procedures.

The sex chromosome trisomy 47, XXY, which is the most common male sex chromosome aneuploidies, can result in a myriad of symptoms, including primary gonadal failure that may cause infertility, gynecomastia associated with hypogonadism, and intellectual disability [2]. Evidence suggested that up to 96% of affected men with nonmosaic KS are azoospermic. However, once labeled infertile with the advent of mTESE and ICSI, around 10% of these patients may eventually father their genetically own child using this revolutionized approach [5, 18]. Given that seminiferous tubules go through extensive fibrotic changes, along with the germ cell undergoing progressive degeneration and depletion starting from midpuberty in these patients, some experts advocate early sperm retrieval since age was proposed as a limiting factor for successful sperm retrieval [19]. A favorable response to hormone optimization treatment with resultant testosterone of 250 ng/dL or higher has also been described as a good prognostic factor for sperm recovery in patients with low baseline testosterone; however, this does not imply that medical treatment leads to a higher sperm retrieval rate [15]. Interestingly, the testosterone gradient between intratesticular fluid and serum was found to exceed 3000 times in men with KS as compared to 30-100 folds for men with normal reproductive potential. This was evidenced by the abnormally high intratesticular testosterone level up to 20000~60000 nmol/L [20]. Significant enrichment in Leydig cell within a testicular biopsy sample can

Table 2: Multivariate logistic regression analyses of factors associated with successful repeat sperm retrieval in men with KS.

	OR (95% CI)	p value
Age (years)	0.809 (0.521–1.257)	0.809
Testis size (mL)	2.449 (0.251–23.934)	0.441
Baseline testosterone (ng/mL) ≥1.5 vs. <1.5	46.969 (1.995–1105.533)	**0.017**
E2 (pg/mL)	1.108 (0.901–1.363)	0.219

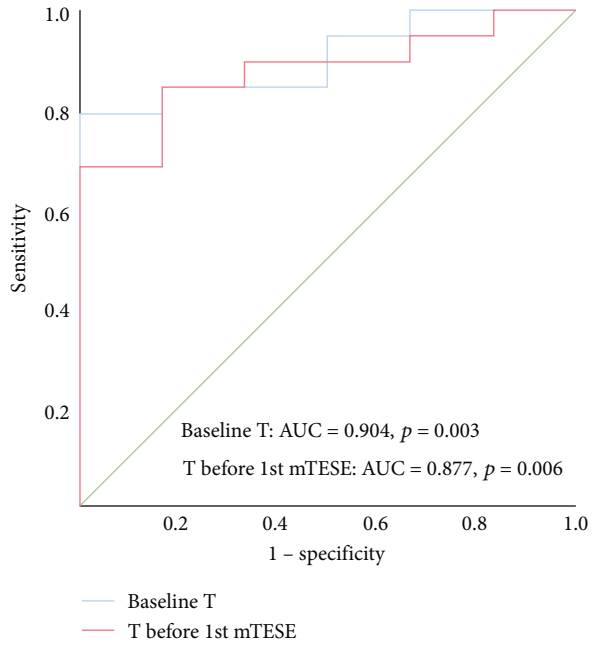

Figure 1: Receiver operating characteristic (ROC) curve showing the ability of baseline testosterone level and testosterone levels before the 1st mTESE attempt to predict successful repeat sperm retrieval.

Table 3: Comparison of repetitive sperm retrieval rate in men with azoospermic KS versus men with idiopathic NOA or spermatogenic failure due to other contributing etiological factors.

	Men with KS ($n = 28$)	NOA men ($n = 128$)	p value
SSR on 2nd attempt	75% (21/28)	89% (114/128)	**0.048**
Age (years)	35.6 ± 4.8	37.4 ± 4.9	0.08
Testis size (mL)	3.6 ± 2.2	10.4 ± 5.4	**<0.001**
Medication usage	64.3% (18/28)	70.3% (90/128)	0.49
Endocrine (baseline)			
FSH (mIU/mL)	29.6 ± 12.5	17.8 ± 10.9	**<0.001**
LH (mIU/mL)	18.3 ± 9.3	7.3 ± 4.5	**<0.001**
Test (ng/mL)	2.3 ± 1.8	3.9 ± 2.1	**<0.001**
Prolactin (ng/mL)	9.1 ± 3.5	12.4 ± 8.3	**0.046**
E2 (pg/mL)	19.5 ± 8.3	20.6 ± 11.7	0.67
Endocrine (before 1st mTESE)			
FSH (mIU/mL)	31.1 ± 14.2	19.9 ± 15.7	0.11
LH (mIU/mL)	16.5 ± 7.8*	12.2 ± 8.3	0.09
Test (ng/mL)	3.2 ± 3.6	7.0 ± 3.0	**<0.001**
Prolactin (ng/mL)	10.4 ± 4.1	9.7 ± 5.5	0.72
E2 (pg/mL)	24.5 ± 14.1	41.2 ± 24.7	**0.004**

*represents LH level in 21 men with KS.

likely explain the excessive production of testosterone, whereas the markedly increased intratesticular/serum testosterone ratio was believed to result from altered testicular vascularization and dynamic perfusion. These all indicated that impairment of testosterone released into the systemic circulation rather than inhibited testicular steroidogenesis was the cause of hypogonadism in men with KS [21].

Our result demonstrated that patients with successful sperm recovery at their 2nd mTESE attempt appeared to have relatively high baseline testosterone and an adequate response to medical treatment. Since better testicular perfusion kinetic, particularly venous blood flow, was associated with relatively higher serum testosterone level [21], we postulated that patients with successful sperm recovery at their 2nd mTESE attempt presented with less disturbed testicular circulation, thus better overall testosterone releasing capability. Additionally, it is plausible that these patients with successful 2nd attempt may have a higher sperm reserve that is sufficient for repeated recovery, which is supported by the fact that improved testicular vascularization correlates with a higher serum testosterone level. Moreover, the development of such a testicular vasculature network represents a microenvironmental niche to establish spermatogenesis [22].

However, there is a paucity of data reporting successful repeated sperm recovery in patients with a prior successful attempt. In two retrospective case series with 103 and 126 patients each, the extraction rate in repeated sperm retrieval procedures in patients with NOA using conventional TESE and mTESE was reported to be 74.7% and 82%, respectively [13, 23]. Of note, repeat mTESE was successful in 75% of attempts in 16 patients as described in one of the aforementioned studies, which is comparable to our results. Additionally, they addressed the issue of predicting the outcome of repeat mTESE attempts and identified FSH and testicular size as predictive factors for successful repeated mTESE. However, the subsequent multivariate analysis in the same study failed to show significant correlation between successful repeat mTESE and these variables [13]. Intriguingly, clinical characteristics present at repeat mTESE instead of baseline clinical data were analyzed in their study, whereas clinically identifiable factors present at baseline and the 1st mTESE attempt were investigated for predicting successful sperm retrieval in the current study.

The findings of the current study have clinical implications such that men with KS and serum testosterone levels below 1.5 ng/mL correspond to a higher risk for unsuccessful repeated sperm harvesting. Hence, they should be properly counseled regarding the sperm recovery rate on repeated mTESE attempts, and the need for sperm cryopreservation on their 1st mTESE procedure with a sperm postthaw survival rate of around 6-30% [12, 13]. Concerning IVF centers dealing with ICSI cycles with fresh sperm, strategies to maximize oocytes numbers ready for fertilization by accumulating oocytes by vitrification should be considered, particularly for those female partners responding inadequately to ovarian stimulation [24], and for patients wherein testicular sperm retrieval may only be successful on the first attempt.

Although some studies have reported higher rates of sperm retrieval than what we found in our study [8, 15], other real-world data from 103 nonmosaic KS patients undergoing testicular sperm extraction has shown retrieval rates between 12.6% and 32.2% [25]. Our study, with a sperm retrieval rate of 42%, is consistent with a recent meta-analysis that reported a range of 38% to 52% [4]. Therefore, our surgical technique is in line with that of other infertility centers, and our findings are likely generalizable to a wider population.

To the best of our knowledge, this is the first study to examine factors associated with repeat mTESE success for patients having sperm harvested on their 1st attempt. However, the study had several important limitations and strengths. Our work was limited by a small sample size and its retrospective nature, which was potentially subject to bias. The results should also be interpreted carefully since all patients were men with KS; therefore, the findings may not be generalizable to men with NOA of other etiologies. However, the hormone milieu inside the testes was not measured in this study as a predictive factor for positive sperm retrieval; hence, it was unclear whether higher ITT led to more advanced spermatogenesis and the optimal ITT levels required for successful sperm retrieval. The complementary information of ITT concentration in men with KS and binary sperm retrieval outcome can help individualize treatment strategies for these individuals in terms of hormone manipulation therapy prior to surgical sperm retrieval.

A major strength of our study was that despite our cohort analysis was based on a single-center sample, it ensured that a standardized sperm retrieval approach and patient evaluation were applied. Furthermore, we retrieve a large cohort of men with NOA undergoing mTESE and assessed the sperm retrieval rate between men with KS against other individuals without gonosomal aneuploidies. This further demonstrated that men with KS have inferior repeated sperm retrieval outcomes.

In summary, azoospermic men with nonmosaic KS presenting with low testosterone level and successful sperm recovery during the first mTESE procedure are unlikely to retrieve sperm on the 2nd mTESE attempt. Hence, these patients should be properly counseled before sperm retrieval, and the remaining testicular sperm used after the ICSI cycle should be cryopreserved and serve as a backup if the 2nd mTESE attempt failed.

Abbreviations

NOA:	Nonobstructive azoospermia
mTESE:	Microdissection testicular sperm extraction
ICSI:	Intracytoplasmic sperm injection
IVF:	In vitro fertilization
WHO:	World Health Organization
FSH:	Follicle stimulating hormone
LH:	Luteinizing hormone
E2:	Estradiol
hCG:	Human chorionic gonadotropin
ROC analysis:	Receiver operating characteristic analysis
PPV:	Positive predictive value
NPV:	Negative predictive value.

Data Availability

Data is available upon request.

Additional Points

Footnote. The authors have completed the observational studies in epidemiology reporting checklist.

Ethical Approval

Approval of the research protocol was obtained from the Institutional Reviewer Board: TPEVGH-IRB 2020-06-016 AC.

Disclosure

The abstract has been presented in 2021 AUA (https://www.auajournals.org/doi/10.1097/JU.0000000000002035.12).

Conflicts of Interest

The author(s) declare that they have no conflicts of interest.

Authors' Contributions

Conception and design were worked on by I-Shen Huang and William J. Huang. Administrative support was conducted by Cheng-Han Tsai. Provision of study materials or patients was executed by I-Shen Huang, Wei-Jen Chen, and William J. Huang. The collection and assembly of data were obtained by Cheng-Han Tsai and I-Shen Huang. Data analysis and interpretation were performed by Cheng-Han Tsai and I-Shen Huang. Manuscript writing was done by all authors. Final approval of the manuscript was given by all authors.

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