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A dual-center study of predictive factors for sperm retrieval through microdissection testicular sperm extraction and intracytoplasmic sperm injection outcomes in men with non-mosaic Klinefelter syndrome

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Purpose: This study evaluated the predictors of sperm retrieval (SR) in non-mosaic Klinefelter syndrome (KS) patients undergoing microsurgical testicular sperm extraction (mTESE). The cutoff values of the predictors of SR and overall pregnancy rates after intracytoplasmic sperm injection (ICSI) were analyzed for the positive SR (PSR) cases.

Materials and Methods: The study was a dual-center retrospective study. Overall 118 patients with KS underwent mTESE between January 2011 and July 2021. Clinicopathological factors including comorbidities, endocrine profiles, and testicular volumes were analyzed.

Results: A total of 58 patients showed PSR (49.2%) and 60 patients (50.8%) had negative SR (NSR). The mean overall age of the patients was 32.5 years. The NSR patients had a significantly greater prevalence of obesity, diabetes mellitus, and cerebrovascular disease. The PSR group had a significantly higher left testis mean volume (p=0.039). The differences between the two study groups regarding follicular-stimulating hormone, luteinizing hormone, and testosterone variations at 1 and 3 months after mTESE were insignificant. Preoperative mean neutrophil-to-lymphocyte ratio was significantly greater in the NSR group (p=0.011), but the platelet-to-lymphocyte ratio showed no significant difference between the two study groups. A live child birth was achieved in 53.4% of the PSR patients. Multivariate logistic analysis showed that total testicular volume >3.93 mL, left testis volume >1.79 mL, and neutrophil-to-lymphocyte ratio ≤1.82 were significantly associated with PSR.

Conclusions: mTESE-ICSI is a feasible method for KS patients to have a child, and total testicular volume, left testis volume, and neutrophil-to-lymphocyte ratio might be predictors of successful SR.

Keywords: Assisted reproductive technology; Azoospermia; Intracytoplasmic sperm injections; Klinefelter syndrome

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INTRODUCTION

Klinefelter syndrome (KS) is the most frequent clinical syndrome of sex chromosomes and affects approximately 1 in 500 to 600 male newborns. The genetic disorder is characterized by sex chromosome aneuploidy of the 47,XXY karyotype [1]. Most cases of KS show a 47,XXY karyotype, but 20% of KS cases have mosaicism (46,XY/47,XXY) or other chromosome aneuploidies such as higher numbers of X chromosomes (48,XXXY) [2,3]. KS is frequently diagnosed in adulthood during a diagnostic workup for male infertility or sexual dysfunction, because KS patients show normal sexual development during the prepubertal period [4]. However, testosterone production begins to decrease in late adolescence and early adulthood, which results in elevated gonadotropin levels, gynecomastia, small firm testis with azoospermia, and various morphologic characteristics including diminished pubic hair development and tall stature [5]. KS patients also have increased risks for glucose intolerance and cerebrovascular disease and higher body mass index (BMI) than genetically normal males [6,7]. Approximately 10% of males with nonobstructive azoospermia have KS, and nearly 90% of adult KS men with the homogeneous 47,XXY karyotype have severe spermatogenesis impairment, whereas mosaic KS men are less severely affected [8]. In addition, the testis tissues of KS males characteristically have a significant decrease of germinal cell numbers during early childhood, and testicular fibrosis with continuous decline of spermatogonia numbers occurs during the peripubertal period [9]. Nevertheless, the development of microsurgical testicular sperm extraction (mTESE), associated with *in vitro* fertilization and intracytoplasmic sperm injection (ICSI) has increased the possibility of pregnancy in the female spouses of KS patients [10]. A previous study reported that successful sperm retrieval (SR) was achieved in 66% of KS men through mTESE [11]. Another recent meta-analytic systematic review analyzed 1,248 male patients with KS and reported a mean SR rate with mTESE of 45%. However, those study results should be interpreted with caution because meta-analysis has limitations associated with the nature of the studies, such as small sample size and unadjusted confounding variables [12]. No consensus currently exists on the specific predictors of successful SR in KS. Some studies have suggested testicular volume and younger age of patients as independent predictors [4,13]. Furthermore, other studies have suggested that sex and reproduction-related hormones such as follicular-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone are not associated with successful SR [14]. Thus, we conducted a dual-center collaborative study primarily to evaluate the predictors of successful SR in non-mosaic KS men undergoing mTESE. Secondarily, we analyzed the values of the predictive factors and the pregnancy rate after ICSI for the positive SR (PSR) cases.

MATERIALS AND METHODS

1. Patients

The study was a dual-center retrospectively designed study of 118 patients with non-mosaic KS who underwent mTESE because of nonobstructive azoospermia confirmed by two consecutive semen analyses according to the World Health Organization (WHO) 2010 criteria. All KS patients underwent mTESE for ICSI between January 2011 and July 2021. This study was approved by the Ethics Committee of the CHA Bundang Medical Center, CHA University School of Medicine (registration no. 2022-06-030). Informed consent was obtained from all KS patients and their female spouses to undertake mTESE and ICSI.

2. Infertility evaluation

The medical history of the KS patients was assessed by use of a self-reported checklist, which included age, comorbidities, and history of genital surgeries, cryptorchidism, and hormone treatment before visiting our clinics. Scrotal ultrasonography was used to analyze testicular volumes and morphology. All patients underwent chromosomal analysis including karyotype analysis, Y-chromosome azoospermia factor (AZF) region microdeletion/partial deletion testing, and cystic fibrosis transmembrane conductor (*CFTR*) gene mutation testing. For each KS patient, venous blood samples were taken between 8 AM and 10 AM. Endocrine profiles, including FSH, LH, testosterone, prolactin, thyroid-stimulating hormone, and triiodothyronine, were measured. Initial laboratory tests, including complete blood cell counts, lipid profiles, and hematologic parameters, were evaluated in all patients. Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were calculated accordingly. BMI was evaluated in every patient. According to the WHO guidelines for Asian men's BMI, measured BMIs were categorized into three classes of normal weight (<23 kg/m^2), overweight (≥23 to <27.5 kg/m^2), and obese (≥27.5 kg/m^2).

3. Procedures of mTESE

All KS patients underwent the surgical procedure of mTESE under general anesthesia. The mTESE was performed by four experienced surgeons having a proficient andrology background and microsurgery specialties. The collected seminiferous tubules were evaluated by at least three

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embryologists for the presence of spermatozoa. The testis tissues harvested for histopathological examinations were analyzed by two senior pathologists. A 3-cm transverse scrotal incision was made to expose the tunica vaginalis. The testis was identified after opening of the tunica vaginalis. With assistance of a surgical microscope (12× to 14× magnification set), a single midtestis transverse incision was performed on the avascular plane of the tunica albuginea anterior surface with a thin scalpel. The hemorrhage produced from small subtunical vessels, which occurred in the peri-incision region, was managed by monopolar electro-cauterization. The testis parenchymal tissue was exposed and dilated seminiferous tubules with relative opacity under the microscope were excised by using sharp micro-scissors and fine surgical forceps. PSR by mTESE was defined as the presence of spermatozoa, including cases in which only immotile sperm were present. The testis-tissue-harvesting process was terminated when PSR was achieved. If no spermatozoa were present, mTESE was performed on the contralateral testis. The tunica albuginea was repaired with 4-0 polyglactin sutures.

The harvested testis tissues were submerged in Bouin's solution and were immediately examined for the presence of spermatozoa and histopathological confirmation. The testis specimens were initially assessed by use of a tissue dispersal process with 24- to 26-gauge fine needles. Then each isolated seminiferous tubule was minced to achieve secondary dispersal. Accordingly, the prepared wet-suspension was thoroughly examined by phase-contrast microscopy (100× to 400× magnification set). Furthermore, the testis tissue specimens were subjected to centrifugation at 1,500 rpm, and the presence of spermatozoa within the centrifuged pellet was carefully reexamined.

4. ICSI and embryo transfer

All procedures required for the female spouses to achieve ICSI and pregnancy, including hormonal stimulation, oocyte retrieval/vitrification, and embryo culture and transfer, were performed as presented in our previous literature [15]. The female partners underwent transvaginal oocyte retrieval by ultrasound-guided needle aspiration as long as the presence of one or more mature follicles with a diameter size ≥17 mm was confirmed. ICSI was performed to mature oocytes by using frozen-thawed spermatozoa obtained from mTESE. Embryo transfer to the uterus was performed at either 3 or 5 days after oocyte retrieval.

5. Statistical analysis

The normal distribution of the variables was analyzed by use of the Shapiro–Wilk normality test. The normally distributed numeric variables of each study group were compared by the Student's t-test, whereas abnormally distributed variables were analyzed by the Mann–Whitney U-test. The Pearson's chi-squared test was performed to evaluate the association between study groups and categorical variables. The best cutoff values of SR predictors by mTESE were evaluated by receiver operating characteristic (ROC) curve analysis. To evaluate the significant predictors of PSR by mTESE, univariate and multivariate logistic regression analyses with odds ratios were undertaken. A p-value ≤0.05 was regarded as statistically significant. The statistical analyses were performed with SPSS version 24.0 (IBM Corp., Armonk, NY, USA) and MedCalc Statistical Software version 20.1.4 (MedCalc Software Ltd, Ostend, Belgium).

RESULTS

1. Baseline clinicopathological characteristics

The study included 118 patients overall. The clinicopathological characteristics of the study cohorts at baseline are summarized in Table 1. Among the 118 KS patients, 58 patients showed PSR (49.2%) by mTESE, whereas the other 60 patients (50.8%) had negative SR (NSR). The results of chromosomal study showed no karyotype abnormalities other than KS, including microdeletion/partial deletion of the Y-chromosome AZF region, *CFTR* gene mutation, chromosomal translocation, or other additional chromosomes, in the entire cohort. The mean age of the overall group of study patients was 32.5 years (range, 22–37 years), whereas the mean ages of the PSR and NSR groups were 31.2 and 33.3 years, respectively. According to the patients' medical histories, the NSR group had a significantly greater prevalence of metabolic and vascular disorders, including diabetes mellitus, hypertension, and cerebrovascular disease, compared with the PSR group (p=0.009). Furthermore, the NSR group had higher mean values of low-density-lipoprotein cholesterol and triglyceride and a lower mean value of high-density-lipoprotein cholesterol than the PSR group. The NSR group had a higher proportion of obese patients compared with the PSR group (p=0.049).

According to the results of preoperative testis ultrasonography, total testis and right testis volumes did not differ significantly between the PSR and NSR groups, but the PSR group had a significantly higher left testis volume than the NSR group (p=0.039). The number of patients with testis microcalcification was significantly greater in the NSR group than in the PSR group (p=0.046). However, the study groups showed no significant difference in varicocele presence (p=0.888). Previous genital surgery history, including previ-

Table 1. Basic characteristics of Klinefelter syndrome patients

Characteristic	Positive sperm retrieval (n=58)	Negative sperm retrieval (n=60)	p-value
Age (y)	31.2±4.9 (22–34)	33.3±5.1 (24–37)	0.420
Disease history			0.009*
Diabetes mellitus	5 (8.6)	15 (25.0)	
Hypertension	7 (12.1)	10 (16.7)	
Cerebrovascular disease	0 (0.0)	2 (3.3)	
Obesity			0.049*
Normal (BMI <23.0 kg/m^2)	22 (37.9)	11 (18.3)	
Overweight (23≤ BMI <27.5 kg/m^2)	30 (51.7)	28 (46.7)	
Obese (BMI ≥27.5 kg/m^2)	6 (10.3)	21 (35.0)	
Lipid profile (mg/dL)			
LDL-cholesterol	123.8±26.1	169.8±30.5	0.033*
HDL-cholesterol	49.6±18.9	30.5±7.3	0.048*
Triglyceride	149.9±135.0	207.9±192.1	0.027*
Testicular volume (mL)			
Right testis	2.0±0.9	1.6±0.9	0.284
Left testis	2.0±0.7	1.3±0.8	0.039*
Total volume (right+left)	4.0±1.7	3.0±1.6	0.069
Presence of testis microcalcification	0 (0.0)	4 (6.7)	0.046*
Previous history of genital surgery			0.513
Previous TESE	7 (12.1)	9 (15.0)	
Hydrocelectomy	2 (3.4)	3 (5.0)	
Spermatocelectomy	0 (0.0)	0 (0.0)	
Orchiopexy due to testicular torsion	0 (0.0)	0 (0.0)	
Orchiopexy due to cryptorchidism	21 (36.2)	23 (38.3)	
Varicocelectomy	19 (32.8)	21 (35.0)	
Presence of varicocele at the time of surgery	11 (19.0)	12 (20.0)	0.888
History of cryptorchidism			
Overall number of undescended testes	21 (36.2)	23 (38.3)	0.812
Direction of undescended testes			0.018*
Unilateral	18 (85.7)	12 (52.2)	
Bilateral	3 (14.3)	11 (47.8)	
Age at orchiopexy (y)			0.134
<1	19 (90.5)	23 (100.0)	
1–2	2 (9.5)	0 (0.0)	
Pre-mTESE hormone level			
TSH (mIU/mL)	2.5±0.8	1.7±1.3	0.105
T3 (ng/mL)	1.2±0.1	1.1±0.3	0.507
Prolactin (ng/mL)	10.1±10.0	12.8±11.1	0.128
FSH (mIU/mL)	31.4±14.9	38.5±13.2	0.113
LH (mIU/mL)	18.8±10.3	21.9±11.4	0.359
Testosterone (ng/mL)	2.3±0.4	2.0±0.5	0.360
Medical treatment prior to mTESE			0.249
Clomiphene citrate	4 (6.9)	8 (13.3)	
Anastrazole	0 (0.0)	0 (0.0)	
hCG	0 (0.0)	0 (0.0)	
Testosterone replacement therapy	0 (0.0)	0 (0.0)	

ous TESE (both conventional TESE and mTESE), hydrocelectomy, and varicocelectomy, did not differ significantly between the two study groups. In terms of cryptorchidism, the number of patients with a history of orchiopexy due to undescended testis (UDT) was similar between the PSR and NSR groups (p=0.812), but the NSR group had a significantly

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Table 1. Continued

Characteristic	Positive sperm retrieval (n=58)	Negative sperm retrieval (n=60)	p-value
Pre-mTESE hematologic level			
WBC (×10^3/μL)	7.1±2.1	7.7±2.3	0.804
Neutrophil	5.0±1.8	6.2±1.8	0.101
Lymphocyte	3.2±1.8	2.8±1.5	0.267
Monocyte	0.6±0.6	0.7±0.3	0.508
Eosinophil	0.2±0.2	0.2±0.2	0.619
Basophil	0.1±0.0	0.1±0.0	0.851
Platelet (×10^3/μL)	234.0±47.9	231.0±50.5	0.382
NLR	1.6±1.7	2.2±1.6	0.011*
PLR	73.8±29.7	82.0±38.1	0.072
Embryo			
Embryo transfer	58 (100.0)	-	-
Number of fertilized embryos	3 (2–7)	-	-
Embryo transfer day			
3rd day	51 (87.9)	-	-
4th day	0 (0.0)	-	-
5th day	7 (12.1)	-	-
Clinical pregnancy			
Yes	42 (72.4)	-	-
No	6 (10.3)	-	-
Biochemical[a]	10 (17.2)	-	-
Number of live births			
0	11 (19.0)	-	-
≥1	31 (53.4)	-	-

Values are presented as mean±standard deviation (range), number (%), mean±standard deviation, or mean (range).
BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TESE, testicular sperm extraction; mTESE, microscopic-TESE; TSH, thyroid-stimulating hormone; T3, triiodothyronine; FSH, follicle-stimulating hormone; LH, luteinizing hormone; hCG, human chorionic gonadotropin; WBC, white blood cell; NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio.
[a]:Biochemical pregnancy: very early miscarriage that occurs in the first few days of pregnancy.
*Statistically significant p-value <0.05.

greater number of patients having a history of bilateral UDT (p=0.018). In both study groups, most of the patients with a history of UDT underwent orchiopexy before 1 year of age (PSR group: 90.5%, NSR group: 100.0%). Although no patients underwent medical or hormonal treatments prior to mTESE during the study protocol, four patients (6.9%) in the PSR group and eight patients (13.3%) in the NSR group received a medical treatment with clomiphene citrate before inclusion in the study.

According to the preoperative hormonal analyses, the NSR group had higher prolactin, FSH, and LH levels than the PSR group, but the difference was statistically insignificant. The preoperative hematologic analysis showed that the NSR group had a significantly higher mean NLR (2.2) compared with the PSR group (1.6) (p=0.011). Mean PLR showed no significant difference between the two study groups (p=0.072).

Transvaginal oocyte retrieval and embryo transfer were performed in all female spouses of the PSR group patients. Among these 58 female spouses who underwent embryo transfer, 42 patients (72.4%) became pregnant, but 6 patients (10.3%) had only biochemical pregnancy. In total, 31 patients (53.4%) of the PSR group had live child births. Among the couples who had live child births, 12 cases had undergone preimplantation genetic testing of the embryo after consultation with a medical geneticist, at the request of both the KS patients and their female spouses. No genetic abnormalities were found in any of the preimplantation genetic testing cases. According to the prenatal screening performed in the female spouses who underwent embryo transfer without preimplantation genetic testing (19 cases), all infants were diagnosed as being genetically normal.

2. Postoperative hormonal variations and histopathological patterns

The post-mTESE hormonal changes and histopathologi-

cal patterns of testis biopsy are demonstrated in Tables 2, 3. At 3 months after mTESE, mean serum testosterone decreased on average by 30.2% and 36.4% from baseline in the PSR and NSR groups, respectively. The differences between the two study groups in terms of FSH, LH, and testosterone changes at 1 and 3 months after mTESE were not significant. Histopathological evaluations of testis biopsy specimens showed that all PSR patients had hypospermatogenesis. In

Table 2. Postoperative serum hormone levels of Klinefelter syndrome patients

Variable	FSH (mIU/mL)	LH (mIU/mL)	Testosterone (ng/mL)
Positive sperm retrieval (n=58)			
Postoperative 1 mo	32.2±13.6 (+2.5)	20.4±11.3 (+8.6)	1.6±0.5 (-30.2)
Postoperative 3 mo	32.9±17.0 (+4.7)	21.2±12.6 (+12.8)	1.4±0.5 (-36.4)
Negative sperm retrieval (n=60)			
Postoperative 1 mo	39.2±16.9 (+2.5)	23.9±13.1 (+8.8)	1.2±0.5 (-38.4)
Postoperative 3 mo	40.3±18.1 (+4.8)	24.9±14.8 (+13.5)	1.2±0.6 (-39.9)
p-value[a]			
Postoperative 1 mo	0.126	0.199	0.381
Postoperative 3 mo	0.120	0.247	0.317

Values are presented as mean±standard deviation (% variation of mean value from baseline) or number only. +Values or -values of %variation in hormones indicate increase and decrease from baseline values, respectively.
FSH, follicle-stimulating hormone; LH, luteinizing hormone.
[a]:p-values were calculated to evaluate the significance of differences between positive and negative sperm retrieval cases regarding mean hormone values at 1 and 3 months after surgery.

Table 3. Histopathologic patterns of Klinefelter syndrome patients

Histopathologic type	Positive sperm retrieval (n=58)	Negative sperm retrieval (n=60)	p-value
Sertoli cell only syndrome	0 (0.0)	47 (78.3)	<0.001*
Tubular hyalinization	0 (0.0)	3 (5.0)	
Hypospermatogenesis	58 (100.0)	0 (0.0)	
Maturation arrest	0 (0.0)	10 (16.7)	
Leydig cell hyperplasia	0 (0.0)	0 (0.0)	

Values are presented as number (%).
*Statistically significant p-value <0.05.

Table 4. Predicted probable cutoff scores and AUC values obtained by ROC analyses

Variable	AUC	Cutoff value	Sensitivity (%)	Specificity (%)	p-value	PPV (%)	NPV (%)
Age (y)	0.571	≤35.12	70.9	55.2	0.198	50.1	77.9
Body mass index (kg/m^2)	0.599	≤28.50	61.7	59.1	0.206	60.5	72.5
Testicular volume (mL)							
Total	0.740	>3.93	82.3	61.2	0.030*	61.7	75.5
Right testis	0.744	>1.60	60.5	87.4	0.028*	77.1	63.8
Left testis	0.752	>1.79	60.3	89.0	0.011*	80.2	64.3
Pre-mTESE hormone level							
Prolactin (ng/mL)	0.749	≤12.65	86.7	53.5	0.072	65.9	81.6
FSH (mIU/mL)	0.635	≤34.08	53.8	66.2	0.135	61.3	53.4
LH (mIU/mL)	0.613	≤24.20	75.6	51.1	0.204	58.4	66.5
Testosterone (ng/mL)	0.625	>2.50	47.1	74.3	0.223	61.7	55.9
Pre-mTESE hematologic indicator							
NLR	0.626	≤1.82	72.3	61.0	0.039*	70.5	60.9
PLR	0.541	≤79.39	44.3	62.8	0.087	43.6	61.4

AUC, area under the curve; ROC, receiver operating characteristic; PPV, positive predictive value; NPV, negative predictive value; mTESE, microscopic-testicular sperm extraction; FSH, follicle-stimulating hormone; LH, luteinizing hormone; NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio.
*Statistically significant p-value <0.05.

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the NSR group, 47 cases (78.3%) showed the Sertoli cell only pattern, 10 cases (16.7%) showed maturation arrest, and 3 cases (5.0%) showed tubular hyalinization.

3. ROC analysis

The optimal cutoff values obtained from the ROC analysis are presented in Table 4. According to the cutoff for discriminating between the PSR and NSR groups, the cutoff value of right testis volume was >1.60 mL (sensitivity 60.5%, specificity 87.4%, p=0.028), with an area under the curve (AUC) of 0.744. For left testis volume, the cutoff value was >1.79 mL (sensitivity 60.3%, specificity 89.0%, p=0.011, AUC 0.752), whereas the cutoff value of total testis volume was >3.93 mL (sensitivity 82.3%, specificity 61.2%, p=0.030, AUC 0.740). The optimal cutoff value for which the NLR can predict PSR in mTESE was ≤1.82 (sensitivity 72.3%, specificity 61.0%, p=0.039), whereas the AUC for the NLR was 0.626.

4. Logistic regression analysis for predictors of SR

Logistic regression analyses were performed to evaluate the predictors for SR, and the results are described in Table 5. Obesity with a BMI greater than 28.5 kg/m^2 was associated with SR failure in univariate analysis (p=0.040) but did not satisfy the independent predictor status in multivariate analysis (p=0.081). Multivariate analysis showed that left testis volume >1.79 mL (p=0.031) was a significant predictor of PSR in mTESE. Right testis volume >1.60 mL was associated with PSR in the univariate analysis (p=0.044) but could not predict SR in the multivariate analysis (p=0.061). A NLR value ≤1.82 was another independent predictor of successful SR in mTESE (p=0.014). The patients' age, presence of testis microcalcification, PNR, and preoperative reproductive

Table 5. Univariate and multivariate logistic regression analysis for predicting positive sperm retrieval in men with Klinefelter syndrome

Variable	Univariate			Multivariate		
	OR	95% CI	p-value	OR	95% CI	p-value
Age (y)						
≤35		Reference			Reference	
>35	0.934	0.910–1.242	0.535	0.919	0.843–1.514	0.759
Obesity (BMI, kg/m^2)						
≤28.5		Reference			Reference	
>28.5	0.920	0.890–0.951	0.040*	0.960	0.885–2.007	0.081
Testicular volume (mL)						
Right testis						
≤1.60		Reference			Reference	
>1.60	1.017	1.033–1.106	0.044*	1.059	0.870–1.204	0.061
Left testis						
≤1.79		Reference			Reference	
>1.79	1.251	1.059–1.513	0.002*	1.101	1.009–1.328	0.031*
Presence of testis microcalcification						
Absent		Reference			Reference	
Present	0.919	0.887–1.122	0.030*	0.935	0.719–1.081	0.127
Pre-mTESE hormone level						
Prolactin (ng/mL)	0.858	0.745–1.019	0.079	0.891	0.701–1.332	0.096
FSH (mIU/mL)	0.916	0.815–1.012	0.075	0.805	0.719–1.030	0.099
LH (mIU/mL)	0.931	0.897–1.141	0.746	0.913	0.902–1.215	0.481
Testosterone (ng/mL)	1.021	0.933–1.238	0.155	1.244	0.990–1.652	0.218
Pre-mTESE hematologic indicator						
NLR						
>1.82		Reference			Reference	
≤1.82	4.658	2.558–8.521	0.001*	4.541	1.993–7.604	0.014*
PLR						
>79.39		Reference			Reference	
≤79.39	1.752	0.973–3.096	0.092	1.819	0.805–4.226	0.110

OR, odds ratios; CI, confidence interval; BMI, body mass index; mTESE, microscopic-testicular sperm extraction; FSH, follicle-stimulating hormone; LH, luteinizing hormone; NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio.
*Statistically significant p-value <0.05.

hormone levels were not associated with SR prediction in mTESE.

DISCUSSION

With the recent advances of mTESE and ICSI, successful SR and becoming a biological father are no longer impossible achievements for KS patients. Although many studies have attempted to evaluate the various clinical predictors for successful SR in KS patients, the results are widely variable and controversial [16]. Thus, we tried to investigate potential predictors of successful SR and pregnancy outcomes in non-mosaic KS patients by analyzing data from two large-scale infertility institutes.

The current study results showed a PSR rate of 49.2% by mTESE and a live birth rate of 53.4% by ICSI among successful SR cases. These values are similar with previous investigations, such as a study by Özkan et al. [13], which analyzed a total of 67 non-mosaic KS patients and reported a PSR rate of 52.2% with a live birth rate of 51.06% among successful SR cases. Previous investigations have shown that the risks for genetic abnormality among babies born to KS couples are equivalent to those of other couples at similar ages [17,18]. Thus, we performed preimplantation genetic testing only when both the KS patient and their partner strongly demanded the testing even after genetic consultation. Because testicular function decreases progressively with age, previous literature proposed younger age as a predictor of successful SR [13,19]. By contrast, some studies including a study by Selice et al. [20] failed to find younger age of KS patient as a significant predictor of successful SR. Although the optimal cutoff age of the patients was calculated by ROC analysis in this study, the patients' age was not associated with PSR in the multivariate logistic analysis.

In the current study, we found that the prevalence of obesity was higher in the NSR patients. A BMI >28.5 kg/m^2 was associated with unsuccessful SR in the univariate logistic regression analysis. Han et al. [21] reported a prevalence of obesity in KS patients of 42.6% when obesity was defined as BMI ≥25.5 kg/m^2. Since this study had an obesity reference of BMI ≥27.5 kg/m^2, the prevalence of obesity of 46.7% in the NSR group is relatively higher than the research results of Han et al. [21].

Another interesting clinical point of this study regarding cryptorchidism in KS patients is that 95.5% of the patients with a history of UDT (42 patients) underwent orchiopexy before 1 year of age. A recent systematic review on cryptorchidism in the general pediatric population recommended undergoing orchiopexy between 6 and 12 months of age to preserve fertility [22]. Our study results could be interpreted as suggesting that performing early orchiopexy alone may not be enough to preserve the fertility of KS patients with UDT. However, our results do not imply that orchiopexy is suitable after 1 year of age in KS patients. Early orchiopexy is strongly recommended for children with KS because they have decreased fertility potential compared with the genetically normal population.

Although some literature, including the study by Ly et al. [23], has failed to define the association between testicular volume and SR rates in KS patients, a recent study of non-mosaic KS patients suggested left testis volume >1.74 mL and right testis volume >1.2 mL as significant cutoff values for successful SR by mTESE [13]. In this study, left testis volume >1.79 mL was significantly associated with successful SR, but right testis volume was not.

In this study, the NLR, a universal inflammatory marker, was an independent predictor of PSR with a cutoff value of ≤1.82, whereas the PLR was not. Although a few studies have reported the association of NLR or PLR with the SR rate in nonobstructive azoospermia patients with a normal karyotype [24,25], to the best of our knowledge, the present study is the first to report the NLR as an independent predictor of SR in KS patients undergoing mTESE.

Accardo et al. [26] reviewed testicular ultrasonography results of 40 consecutive KS patients and reported that 8 patients (20%) showed the presence of microcalcification. Testicular microliths are mineral composites of hydroxyapatite within the seminiferous tubules [27], but their clinical significance was unable to be defined in this study because of the small sample size.

Many researchers have attempted to identify the hormonal predictors of SR failure in KS patients, and a study suggested that high FSH and LH levels are associated with unsuccessful SR in patients with nonobstructive azoospermia [28]. By contrast, another study failed to show that FSH, LH, and testosterone levels are predictors of SR [13], which is similar to our study results.

The limitations of this study include the relatively small sample size. Because of the retrospective nature of the investigation, the study was not devoid of selection bias. The age range of the patients was narrow, and the ethnicity of the cohort was homogeneous, which implies our study results may not be applicable to KS patients of various ages and ethnic backgrounds. Thus, we believe further investigations with larger sample sizes are required to make definite confirmation of the predictors of SR in KS patients. Nevertheless, this study contains the strength of the comprehensive evaluation of various confounders, including hormones, his-

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tory of UDT and testicular microcalcification, lipid profiles and obesity, underlying disease history, testicular volumes, and the NLR.

CONCLUSIONS

This study performed mTESE in non-mosaic KS patients and achieved a PSR rate of 49.2%. The live birth rate was 53.4% after ICSI in the PSR cases. The potential predictors of PSR were the NLR and left testis volume. To our knowledge, this is the first study to identify the NLR, which is a systemic inflammation parameter, as an independent predictor of SR in KS patients undergoing mTESE. mTESE-ICSI is a feasible method for KS patients to have a child.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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AUTHORS' CONTRIBUTIONS

Research conception and design: Young Dong Yu. Data acquisition: Jong Hyeun Baeck, Tae Jin Kim, Tae Heon Kim, Seung-Ryeol Lee, Dong Soo Park, Ji Eun Shin, Hwang Kwon, and Young Dong Yu. Statistical analysis: Dong Hyeon Lee and Young Dong Yu. Data analysis and interpretation: Dong Hyeon Lee and Young Dong Yu. Drafting of the manuscript: Jong Hyeun Baeck and Young Dong Yu. Critical revision of the manuscript: Seung-Ryeol Lee and Young Dong Yu. Administrative, technical, or material support: Dong Hyeon Lee and Young Dong Yu. Supervision: Seung-Ryeol Lee and Young Dong Yu. Approval of the final manuscript: all authors.

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