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ORIGINAL ARTICLE

Male Infertility

Sperm retrieval success and testicular histopathology in idiopathic nonobstructive azoospermia

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Prior studies have investigated sperm retrieval rates in men with nonobstructive azoospermia (NOA) secondary to specific etiologies, yet most cases of NOA are idiopathic. We compared sperm retrieval rates and testicular histopathology in idiopathic NOA (iNOA) and nonidiopathic NOA (niNOA). We performed a retrospective review of men with NOA who underwent microdissection testicular sperm extraction (microTESE) between 2000 and 2016. Men with no history of malignancy or cryptorchidism and negative genetic evaluation were considered idiopathic. Multivariable regression determined the association between idiopathic etiology and primary outcomes of sperm retrieval and active spermatogenesis on histopathology. Among 224 men, 86 (38.4%) were idiopathic, 75 (33.5%) were nonidiopathic, and 63 (28.1%) did not undergo genetic testing. Median age and serum testosterone were higher among iNOA or no testing versus niNOA. Median follicle-stimulating hormone (FSH) was lower among iNOA or no testing versus niNOA. A higher proportion of iNOA or no testing versus niNOA had a clinical varicocele. Sperm retrieval rates were similar between iNOA, niNOA, and no testing (41.8% vs 48.0% vs 55.6%, respectively; $P = 0.255$). Active spermatogenesis was seen in a higher proportion of iNOA or no testing versus niNOA (31.4% and 27.0% vs 16.0%, $P = 0.073$). On multivariable analysis, iNOA was not associated with sperm retrieval or spermatogenesis ($P = 0.430$ and $P = 0.078$, respectively). Rates of sperm retrieval and spermatogenesis on testis pathology were similar in men with iNOA and niNOA. These data will be useful to clinicians in preoperative counseling for men with NOA and negative genetic evaluation.

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INTRODUCTION

Infertility affects 8%–12% of couples worldwide, and 40%–50% of infertile couples have a male factor.¹ Approximately 5%–10% of these men are azoospermic, more than half of whom have nonobstructive azoospermia (NOA).^{2,3} Men with NOA may have primary spermatogenic failure due to a specific, identifiable cause such as genetic (Y chromosomal microdeletions and Klinefelter syndrome), congenital (cryptorchidism), postinfectious (mumps orchitis), gonadotoxic (radiotherapy, chemotherapy), or traumatic insult.⁴ However, a substantial proportion of men with NOA have no identifiable etiology, rendering these patients idiopathic.⁵

Regardless of etiology, microdissection testicular sperm extraction (microTESE) has emerged as the gold standard treatment for men with NOA. Testicular heterogeneity in these patients offers the possibility of sperm retrieval from small foci of active spermatogenesis, which can facilitate biological fatherhood through *in vitro* fertilization (IVF) with intracytoplasmic sperm injection (ICSI). Given the potential morbidity of a microTESE procedure, many studies have aimed to identify factors predictive of sperm retrieval in men with NOA, an inherently heterogeneous group, to facilitate preoperative patient counseling regarding expectations and postoperative outcomes.^{6–8}

While clinical and laboratory variables such as testicular volume, preoperative follicle-stimulating hormone (FSH), and testosterone are not strongly associated with successful sperm retrieval, multiple studies have characterized the prognostic significance of certain genetic abnormalities.^{9–12} Sperm retrieval rate (SRR) can be reliably predicted in men with specific etiologies of NOA such as karyotype abnormalities (*i.e.*, Klinefelter syndrome) or azoospermic factor c (*AZFc*) deletions, which are associated with better SRR. In contrast, other genetic mutations such as *AZFa* and *AZFc* deletions harbor very poor prognosis.^{13–16} These data are useful to inform patients regarding the potential benefit of microTESE, an important element of preoperative counseling.

However, a substantial proportion of men with NOA have an idiopathic etiology (iNOA).⁵ While the rates of iNOA vary according to the definition of idiopathic across the literature, even when the most expansive definitions are utilized, approximately 22% of men with NOA will be idiopathic.¹⁷ Given the paucity of data characterizing SRR in men with iNOA, there is an inability to appropriately counsel these men regarding potential outcomes of microTESE. We sought to determine the SRR for men with iNOA at a single institution and identify prognostic factors associated with improved outcomes.

PATIENTS AND METHODS

Patient population

We performed a retrospective review of all men with NOA who underwent microTESE between 2000 and 2016 at Northwestern Memorial Hospital (Chicago, IL, USA). Semen analysis was performed according to the World Health Organization (WHO) 2010 guidelines.¹⁸ A 5 µl specimen was placed in 20-µl chamber to examine the specimen at ×200 magnification. A minimum of two fields were examined for each specimen. If no sperm was visualized, the specimen was further centrifuged and the pellet was examined for sperm at ×200 magnification. Nonobstructive etiology was determined according to the hormonal evaluation based on previously suggested cutoffs.¹⁹

Men were classified as iNOA if they had no history of malignancy or cryptorchidism, and genetic evaluation was negative for Y-chromosome and complete karyotype (46 chromosome) abnormalities. Men who did not undergo genetic evaluation were considered separately. Demographic and clinical variables including age, preoperative testosterone, and FSH were abstracted.

All microTESE procedures were performed by a single surgeon. The procedure has been described previously.²⁰ In brief, a mid-pole transverse testicular incision in the tunica albuginea was utilized to open and subsequently expose the testicular parenchyma. Using the operating microscope (Leica Microsystems M500-N, Wetzlar, Germany), systematic microdissection was performed, and targeted tissue was processed for intraoperative microscopic evaluation with a phase-contrast microscope (Leica Microsystems DMLB) at ×200 and ×400 magnification. When spermatozoa were identified intraoperatively, further tissue processing was performed before cryopreservation in the laboratory. All men underwent intraoperative testis biopsy for formal pathological analysis. Pathological diagnosis was determined by an experienced genitourinary pathologist and categorized as active spermatogenesis, hypospermatogenesis, maturation arrest (MA), Sertoli cell-only pattern, or atrophy/fibrosis.

Primary outcome and statistical analyses

The primary outcome was successful sperm retrieval. The secondary outcome was spermatogenesis (active or hypospermatogenesis) on testis histopathology. The Chi-square test was used to determine the association between etiology of NOA and the outcomes of interest. Multivariable logistic regression was used to determine the factors associated with sperm retrieval and spermatogenesis on histopathology. Statistical significance for all testing was determined at a $P = 0.05$. All statistical analyses were performed using Stata version 13.0 (StataCorp, College Station, TX, USA). Northwestern University Institutional Review Board (IRB) approval was obtained with waiver of consent given the low risk of harm from study participation (protocol

#STU00206101). A waiver of written consent and Health Insurance Portability and Accountability Act (HIPAA) authorization was also granted for retrospective analysis.

RESULTS

Patient characteristics

The total cohort consisted of 224 patients with NOA; 75 (33.5%) nonidiopathic NOA (niNOA), 86 (38.4%) iNOA, and 63 (28.1%) did not have genetic evaluation and thus could not be considered in either category. niNOA etiologies included history of malignancy ($n = 30$, 40.0%), genetic abnormality ($n = 28$, 37.3%), and history of cryptorchidism ($n = 15$, 20.0%). Two patients (2.7%) had both cryptorchidism and malignancy.

Patient characteristics are presented in **Table 1**. Median age was significantly higher among men with iNOA (34 years, interquartile range [IQR]: 31–39 years) or no testing (35 years, IQR: 32–38 years) versus niNOA (32 years, IQR: 29–35 years), $P = 0.001$. Median serum testosterone (ng dl⁻¹) was higher among men with iNOA (365, IQR: 307–477) or no testing (378, IQR: 320–464) versus niNOA (343, IQR: 240–421) ($P = 0.016$). Median FSH (in mIU ml⁻¹) was lower among men with iNOA (20.2, IQR: 10.3–26.8) or no testing (16.2, IQR: 6.2–24.9) versus niNOA (24.1, IQR: 15.1–31.2) ($P = 0.003$). A higher proportion of men with iNOA or no testing versus niNOA had a clinical varicocele (15.1% and 23.8% vs 5.3%, $P = 0.008$).

Sperm retrieval and testicular histopathology

Sperm retrieval rates were similar among men with iNOA, niNOA, and no testing (41.8% vs 48.0% vs 55.6%, respectively; $P = 0.255$). On multivariate analysis, none of the aforementioned variables – etiology (niNOA as reference; $P = 0.430$ for iNOA and $P = 0.374$ for no testing), testosterone ($P = 0.267$), FSH ($P = 0.796$), and varicocele ($P = 0.982$) – were associated with sperm retrieval.

Distribution of testis histopathology is presented in **Figure 1**, and sperm retrieval rates according to the etiology of NOA and testis histopathology are presented in **Figure 2**. Spermatogenesis was seen in a higher proportion of men with iNOA or no testing versus niNOA, approaching but not reaching significance (31.4% and 27.0% vs 16.0%, $P = 0.073$). However, on multivariable analysis, etiology of NOA was not associated with spermatogenesis on testicular histopathology (niNOA as reference; $P = 0.078$ for iNOA and $P = 0.237$ for no testing).

DISCUSSION

Before the development of TESE, NOA was a clinical condition with limited treatment options. The advent of microTESE, along with the arrival of IVF/ICSI, rendered it possible for men with NOA to father biological children.²⁰ Since then, microTESE has emerged as the gold standard for the treatment of men with NOA.

Table 1: Patient characteristics of men with nonobstructive azoospermia who underwent microdissection testicular sperm extraction

Characteristics	Nonidiopathic	Idiopathic	No testing	<i>P</i>
Total, <i>n</i> (%)	75 (33.5)	86 (38.4)	63 (28.1)	
Malignancy	30 (40.0)	0 (0)	0 (0)	<0.001
Cryptorchidism	15 (20.0)	0 (0)	0 (0)	<0.001
Genetic abnormality	28 (37.3)	0 (0)	–	<0.001
Multiple	2 (2.7)	0 (0)	0 (0)	0.135
Age (year), median (IQR)	32 (29–35)	34 (31–39)	35 (32–38)	0.001
Serum T (ng dl ⁻¹), median (IQR)	343 (240–421)	365 (307–477)	378 (320–464)	0.016
Serum FSH (mIU ml ⁻¹), median (IQR)	24.1 (15.1–31.2)	20.2 (10.3–26.8)	16.2 (6.2–24.9)	0.003
Varicocele, <i>n</i> (%)	4 (5.3)	13 (15.1)	15 (23.8)	0.008

[†]Chi-squared test comparing all three groups, where applicable. IQR: interquartile range; T: testosterone; FSH: follicle stimulating hormone; –: not applicable

Owing to the significant potential morbidity of microTESE, it is critical that men be informed of the likelihood of success to determine the tradeoff between the potential risks and benefits of the procedure. Testicular pain, injury, and, in rare circumstances, atrophy are all potential complications. Schlegel and Su²¹ demonstrated that 82% of men had ultrasonographic evidence of inflammation or hematoma formation at the site of biopsy 3 months after conventional TESE, two of whom had impaired testicular blood flow. While hematoma is less likely with microTESE, microTESE is still associated with a risk of bleeding and damage to testicular tissue.²² Postoperative hypogonadism is also an important concern as a recent systematic review found that a significant, albeit transient, drop in total testosterone occurs within 6 months after the procedure.²³ Weighing these complications against the potential benefit of microTESE is an important aspect of preoperative counseling. For example, men with *AZFa* and *AZFB* deletions, in whom successful sperm retrieval has never been reported, would not be good candidates for surgery.

We found that SRR in iNOA was similar to those in niNOA. For comparison, we abstracted estimates of SRR for iNOA from other series in the literature, many of which did not expressly report SRR for the iNOA cohort (Table 2). The mean SRR across 15 studies was 37.9%

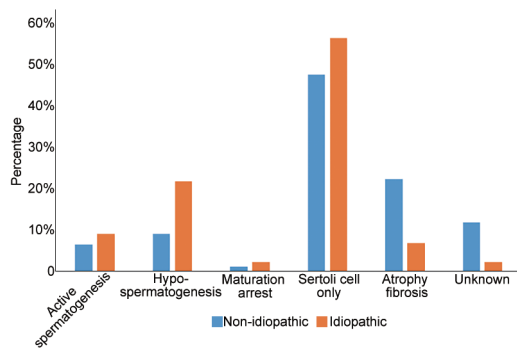


Figure 1: Testicular histopathology in men with azoospermia who underwent microdissection testicular sperm extraction.

(range 9.1%–50.1%), which is similar to the present findings.^{5,9,16,24–35} The heterogeneity among these studies can be explained by the inconsistent definition of iNOA. Only seven studies evaluated patients for Y chromosome microdeletions, while the remaining studies identified patients with 46,XY karyotypes as idiopathic.^{5,16,28,29,33–35} We utilized a more stringent definition of iNOA, including only those men who had both karyotype and Y chromosome microdeletion testing, which must be considered when using these data to counsel patients in the preoperative setting.

These data can be useful in preoperative counseling before microTESE. In our cohort, over one-third of men did not have an identifiable etiology of NOA, which is likely an underestimate owing to the substantial proportion of men without genetic testing. For these men with iNOA, the present data can be useful in setting expectations regarding success of sperm retrieval with microTESE.

Beyond specific etiologies for NOA, testicular histology is perhaps the best predictor of successful or unsuccessful microTESE.^{36–39} While preoperative testicular biopsy in clinical practice has limited utility, the presence of active spermatogenesis on intraoperative biopsy is strongly correlated with successful sperm extraction.⁶ In our cohort, men with

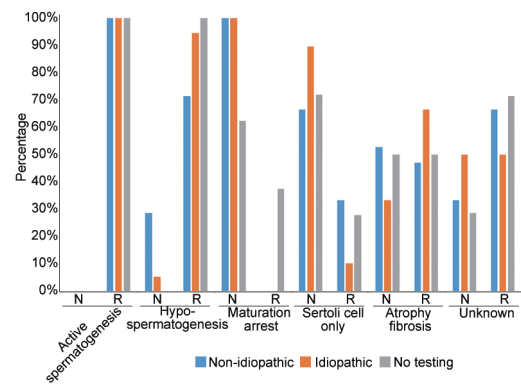


Figure 2: Success of sperm retrieval according to etiology of nonobstructive azoospermia and testicular histopathology. N: no retrieval; R: retrieval.

Table 2: Rates of sperm retrieval in idiopathic nonobstructive azoospermia as reported in the literature

Author	Year	Patients (n)	Retrieval rate (%)	Testing and definition for idiopathic		
				XXY	Y microdeletion	Other causes
Tsujimura and Matsumiya ⁹	2004	100	41	x	–	–
Ishikawa <i>et al.</i> ²⁴	2009	100	41	x	–	–
Yarali <i>et al.</i> ²⁵	2009	113	44	x	–	–
Stahl <i>et al.</i> ¹⁶	2010	385	48.8	x	x	–
Bakircioglu <i>et al.</i> ²⁶	2011	379	50	x	–	–
Arafa <i>et al.</i> ^{28*}	2015	97	45.4	x	x	x
Arafa <i>et al.</i> ^{28**}	2015	22	9.1	x	x	x
Sabbaghian <i>et al.</i> ²⁷	2014	537	22.2	x	–	–
Enatsu <i>et al.</i> ²⁹	2016	194	21.1	x	x	–
Alfano <i>et al.</i> ⁵	2017	47	49	x	x	x
Iwatsuki <i>et al.</i> ³¹	2017	172	26.2	x	–	–
Takeda <i>et al.</i> ³²	2017	144	27.1	x	–	–
Binsaleh <i>et al.</i> ³⁰	2017	138	41.3	x	–	–
Klami <i>et al.</i> ³³	2018	65	31	x	x	x
Ozkanli <i>et al.</i> ³⁴	2018	70	47.1	x	x	x
Johnson <i>et al.</i> ³⁵	2019	423	50.1	x	x	–

*Nonfamilial idiopathic NOA; **familial idiopathic NOA. x: patients tested and evaluated for this condition were included in the study and considered “idiopathic”; –: patients were not tested or evaluated for this condition in the study when defining “idiopathic”; NOA: nonobstructive azoospermia

iNOA had a higher incidence of active spermatogenesis compared to niNOA, although the difference was not statistically significant (31.4% vs 16.0%, $P = 0.07$). While it remains impractical to perform a diagnostic biopsy before a microTESE when preoperative evaluation is sufficient for accurate diagnosis, diagnostic biopsy at the time of the initial microTESE for iNOA may provide prognostic evidence as to whether repeat microTESE would be reasonable.¹⁹

Our study must be interpreted within the context of limitations in study design. First, this is a retrospective study that is subject to the typical biases of such analyses. Second, we did not exclude patients with varicoceles from the analysis, nor did we consider varicocele as a distinct etiology for NOA. That is, men with varicocele could be considered idiopathic, despite the presence of pathology that could impair semen parameters. While there are limited data to suggest that varicocele repair in men with NOA can improve SRR, this does not necessarily imply that varicocele alone is a sufficient etiology for NOA in the absence of other identifiable abnormalities.⁴⁰ Nonetheless, a greater proportion of men in the iNOA and no testing cohorts had varicoceles compared with the niNOA cohort, which does raise the possibility of varicocele as a distinct etiology for NOA that should be considered separately. Third, as is the case at most institutions, we did not routinely perform tissue digestion in cases where no spermatozoa were identified intraoperatively, which in one retrospective series led to additional sperm identification in 7% of cases.⁴¹ However, a thorough, microdissection approach was utilized in all cases with meticulous intraoperative assessment of all tissue samples.

CONCLUSIONS

In a retrospective analysis of men with NOA who underwent microTESE, the rates of sperm retrieval and spermatogenesis on testicular pathology were similar in men with iNOA and niNOA. These data will be useful to patients and clinicians in preoperative counseling for men with NOA and negative genetic evaluation.

AUTHOR CONTRIBUTIONS

AD performed the literature review and synthesis, and helped to draft the manuscript. JAH helped with conception of the study, performed the statistical analysis, and helped with the initial draft and critical revision of the manuscript. ALDB and MP assisted with the literature synthesis, data interpretation, and critical revision of the manuscript. JW helped with conception of the study, data interpretation, and critical revision of the manuscript. MKK helped with statistical analysis, data interpretation, and critical revision of the manuscript. REB helped the conception of the study, oversight, and critical revision of the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interest.

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