Endocrine Tests and/or Testicular Volume Are Not Predictive of Successful Sperm Retrieval by Multiple Testicular Sperm Extraction in Non-Obstructive Azoospermia

Minh Tam Le (leminhtam@huemed-univ.edu.vn)
1 Center for Reproductive Endocrinology and Infertility, Hue University of Medicine and Pharmacy, Hue University, 06 Ngo Quyen Street, Hue, Vietnam. 2 Department of OBGYN, Hue University of Medicine and Pharmacy, Hue University, 06 Ngo Quyen Street, Hue, Vietnam. https://orcid.org/0000-0001-6225-3108

Thanh Tam Thi Nguyen
Department of Diagnostic Imaging, Hue University of Medicine and Pharmacy, Hue University, 06 Ngo Quyen Street, Hue, Vietnam https://orcid.org/0000-0003-2463-3040

Dac Nguyen Nguyen
Department of OBGYN, Hue University of Medicine and Pharmacy, Hue University, 06 Ngo Quyen Street, Hue, Vietnam https://orcid.org/0000-0002-0406-1711

Nhu Quynh Thi Tran
Center for Reproductive Endocrinology and Infertility, Hue University of Medicine and Pharmacy, Hue University, 06 Ngo Quyen Street, Hue, Vietnam. https://orcid.org/0000-0002-6252-8447

Quoc Huy Vu Nguyen
Department of OBGYN, Hue University of Medicine and Pharmacy, Hue University, 06 Ngo Quyen Street, Hue, Vietnam https://orcid.org/0000-0002-4744-7059

Research Article

Keywords: testicular volume, follicular stimulating hormone, sperm retrieval, azoospermia

Posted Date: July 24th, 2020

DOI: https://doi.org/10.21203/rs.2.11012/v2

License: ☑️ This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

**Objectives:** This study aimed to determine the role of pre-surgical markers in the prediction of sperm retrieval in infertile Vietnamese men with azoospermia.

**Patients and Methods:** Retrospective descriptive analysis of 136 infertile men with azoospermia, examined from August 2014 to July 2018. Patients underwent stepwise surgical sperm retrieval via percutaneous epididymal sperm aspiration, testicular sperm aspiration then multiple testicular sperm extraction in up to 3 locations until sperm were detected. Factors were analyzed to determine the prediction of sperm retrieval.

**Results:** The overall success rate of sperm retrieval was 49.3% including 88.3% and 18.4% in the OA and NOA group, respectively. The results of sperm retrieval were significantly associated only with the OA and NOA group, not with endocrine test or testicular volume. We found no significant difference in the endocrine test and testicular volume’s result between successful and unsuccessful sperm retrieval in either group.

**Conclusions:** Neither an endocrine test nor testicular volume should be used for predicting the results of surgical sperm retrieval in infertile Vietnamese males with azoospermia.

Introduction

Azoospermia is one of the major reproductive disorders. It is reported in approximately 1% of males and 10–15% of infertile males. [1,2] It includes non-obstructive azoospermia (NOA) and obstructive azoospermia (OA). [3] OA is less common than NOA and occurs in 15 to 20% of men with azoospermia. [4] NOA indicates impaired sperm production of the entire testis, although it has been observed that focal normal spermatogenesis can occur in 50–60% of men with NOA.[5]

In fact, abnormal spermatogenesis may be caused by any factor related to the hypothalamic–pituitary–testicle axis, including the effects of follicle stimulating hormone (FSH), luteinizing hormone (LH), or testosterone on the function of the testis. [6] Due to the pathophysiology, NOA and OA are managed differently. While sperm retrieval directly from the epididymis or testis had a dramatically high success rate in OA patients, NOA patients may have fewer chances to be father via surgery which may achieve successful sperm extraction. [7] With the achievement of assisted reproductive technologies (ART), almost every infertile man with azoospermia is a candidate for surgical sperm retrieval through percutaneous epididymal sperm aspiration (PESA), testicular sperm aspiration (TESA), or multiple testicular sperm extraction (TESE) to extract sperm for treatment by the intracytoplasmic sperm injection (ICSI). Unfortunately, mature sperm are not found in certain cases and these invasive procedures may be futile. Therefore, it is important to have certain noninvasive testing that is able to predict spermatogenesis. Currently, an accurate means of predicting spermatogenesis and sperm retrieval success do not exist.
Testicular function can be assessed by clinical examination, hormonal tests, semen analysis, scrotal ultrasound and biopsy of the testis. [8] Based on the activities on the hypothalamic–pituitary–testicle axis, serum FSH and LH have been reported as important preoperative markers reflecting testicular function. [6] Serum FSH and LH concentration is inversely related to the sperm retrieval rate. [9] In a recent meta-analysis reported by Qi Yang et al. FSH had a moderate value in independently predicating successful sperm retrieval rates in men with NOA (area under curve >0.7). Therefore, they suggested that more detailed diagnostic testing should be examined in the future in order to confirm the diagnostic value of other noninvasive parameters. [10]

Scrotal ultrasound has become a more common method in the assessment of the testicle. The sensitivity and specificity of varicocele detection approaches 100% with color Doppler ultrasonography. [11] Some studies have shown that measuring the testicular volume by ultrasonography is more accurate than by the Prader orchidometer and the formula $L \times H \times W \times 0.71$ is the most accurate for calculation of the testicular volume. [12] Testicular volume has been noted to have a direct correlation with semen profiles [13, 14] as well as the production ability of sperm in males. [15]

It is reported that the recovery of spermatozoa is successful in approximately 50% of cases and therefore the prediction of the success rate of sperm retrieval is necessary before attempted surgery. [16] By using current popular method in sperm retrieval, micro TESE, no single clinical finding or test that is able to predict successful sperm retrieval precisely has been found. Furthermore, a variety of optimal cut-off values for testes volume and endocrine profiles for infertile males have been reported. [9, 17-22] This is possibly due to the effects of the study population and ethnicity. [23] This study aimed to determine the role of pre-surgical markers in the prediction of sperm retrieval in infertile men with azoospermia in a Vietnamese population.

**Patients And Methods**

*Patient selection and evaluation*

This was a retrospective cohort design which included 136 infertile males with azoospermia on semen analysis based on the World Health Organization (WHO) 2010 standard criteria (i.e. no sperm after centrifugation of total ejaculate in at least two semen analyses). The men presented at the Hue Center for Reproductive Endocrinology and Infertility (HUECREI), Hue University Hospital from August 2014 to July 2018. Azoospermia is diagnosed by the complete absence of spermatozoa in the semen after two different centrifuged samples (3000 x g for 15 minutes). [3] Azoospermia patients with $FSH \geq 7.6$ mIU/ml with testicular long axis $\leq 46$ mm were categorized into the NOA group. The remaining patients belonged to the OA group. [24] Patients with acute systemic diseases, acute urinary tract infection, hepatic function disorders, malignant diseases, retrograde ejaculation, and hypogonadotropic hypogonadism were excluded. All patients’ general characteristics including age, geography, education, occupation, clinical history, physical examination, infertility type, infertility duration, history of mumps, history of surgery on reproductive urinary tract and testicular density were recorded.
**Scrotal ultrasound technique**

Testicular ultrasound was performed to evaluate testicular echogenicity and homogeneity in 2D Ultrasound and the presence of varicocele was assessed in color Doppler ultrasound (CDUS). Ultrasounds were performed in a warm room with the patient in a supine position and the penis resting on the lower abdomen. The testes were examined in at least two planes, along the long and transverse axes and each testicle was measured in three dimensions (length, width, height). The volume was then calculated by the Lambert formula $V = 0.71 \times L \times W \times H$. We used a high frequency linear probe (7.5 MHz) for both power and spectral CDUS with Samsung Medison R5, Korea. Assessment of the varicocele was performed by measuring the largest diameter and reflux in the vessels before and after the Valsalva maneuver. Color Doppler parameters were optimized for the evaluation of slow flow (wall filter at minimum levels, gain at the maximum level permitted by the presence of artifacts, and elective focus on the region of interest) and standardized using a simplified version of the Sarteschi classification. [25] All examinations were performed by the same ultrasonographer.

**Hormone assays**

Venous blood samples were collected on the day of recruitment after the second semen analysis confirmed azoospermia. LH, FSH, and total testosterone levels were assessed by electrochemiluminescence (ECLIA) using Elecsys and Cobas E immunoassay analyzers (Cobas 4000/6000, Roche Diagnostics, Indianapolis, USA). The FSH's inter-assay coefficient of variation (inter-assay CV) is 3.95%, LH's is 2.1%, prolactin's is 3.8% and testosterone's is 3.35%. All measurements were performed at the Hue University Hospital laboratory as per manufacturer instructions.

**Surgical sperm retrieval procedures**

All patients underwent surgical sperm retrieval via three techniques: percutaneous epididymal sperm aspiration (PESA), testicular sperm aspiration (TESA), and then multiple testicular sperm extraction (TESE) consecutively, up to three locations until sperm was found. Each procedure was carried out on the right and then the left testis in turn. The scrotum was cleaned with antiseptic and then washed with saline to eliminate any residual antiseptic. In the PESA procedure, the head of the epididymis was palpated and then stabilized between the thumb and forefinger. It was then punctured, directly through the scrotal skin. The needle was gently advanced into the epididymis. The aspirated fluid was checked for sperm. TESA was performed with a 20G butterfly needle in 3 different positions on the testis while applying suction with a 1 ml Becton–Dickinson syringe. If both the TESA and PESA failed to retrieve sperm, a TESE was then performed by making an incision (3 cm), just above each side of the testis, resecting up to three pieces of testicular tissue and examining it under a microscope at x300 magnification to detect spermatozoa. The surgical procedure was stopped at any step once the lab staff found sperm successfully or after unsuccessful TESE on both sides, that is, if absolutely no sperm was found.

**Statistical analysis**
Statistical analysis was performed by Stata version 15.0. Patients with successful versus unsuccessful sperm recovery were compared with a Chi-square test, and an independent sample T-test. If the data were not normally distributed, then the Wilcoxon rank sum test (Mann–Whitney U test) was used. The normality distribution of the data was tested by the Shapiro–Wilk normality test. The threshold for statistical significance was confirmed as $P<0.05$.

**Results**

A total of 136 men with azoospermia, including 60 OA and 76 NOA patients, were divided into two groups according to the results of surgery. Surgical sperm retrieval was successful in 67 of 136 patients (49.3%). The age range of the successful sperm retrieval group was 26–70 years (mean of 37.4 ± 10.1 years) whereas the age range was 24–50 years (mean 33.8 ± 5.7 years) for the patients in the unsuccessful sperm retrieval group.

The general and clinical characteristics of the OA and NOA groups are presented in Table 1. There was no significant difference between the 2 groups regarding the patient’s age, geography, education, occupation, infertility type, infertility duration, history of surgery and/or mumps. There were significant differences in testicular density between the 2 groups ($P<0.001$).

Table 2 describes the baseline demographics of successful and unsuccessful semen retrieval in the OA and NOA groups. In terms of the OA group, there was no significant difference in the clinical characteristics between the two groups. The primary infertile patient’s rate of unsuccessful sperm retrieval group in the NOA group was much higher than that of the successful sperm retrieval group (98.4% vs. 78.6%, $P=0.018$). Other characteristics were not significant between the two groups.

The comparisons of endocrine tests and testicular volume between two groups according to the sperm retrieval outcomes are shown in Table 3. In both the NOA and OA groups, men with successful sperm retrieval had a greater mean testicular volume than the unsuccessful sperm retrieval men, however the difference was not statistically significant (NOA group: 5.68±2.37 vs. 4.46±2.83, $P=0.138$; OA group: 14.90±7.47 vs. 11.14±5.94, $P=0.208$). The differences in the endocrine tests between the two groups was also not significant in terms of LH, FSH and testosterone ($P>0.05$).

**Discussion**

This study investigated the predictive value of ultrasound parameters and hormone levels for successful sperm retrieval in azoospermia. The sperm retrieval rate in this study was 49.3% which was similar to the results of Salehi et al. [16]. Ramasamy et al. reported sperm retrieval rates of 32% with conventional TESE and 57% with microsurgical testicular sperm extraction (Micro-TESE). [26] Similarly, Ishikawa T et al. showed that the sperm retrieval rate was 42% when they performed micro-TESE for NOA patients. [27] The various results between these studies could be partially explained by the various characteristics of the patients (OA, NOA) and the sperm recovery methods (TESA, PESA, TESE or Micro-TESE).
FSH is a glycoprotein that stimulates Sertoli function and some studies considered that it is associated with androgen production, which is necessary for fertility. Azoospermia has been a consistent finding in men with loss of function mutations in FSHβ. [28, 29] Serum FSH could predict the existence of sperm which could be retrieved by conventional TESE. [27] Elevated plasma levels of FSH above 19.4 mIU/mL can suggest no spermatogenesis and hence an unsuccessful sperm retrieval. [19] However, the predictive value of FSH remains contentious, with certain studies showing that FSH has a low predictive value for sperm retrieval. Although serum FSH is useful marker for the evaluation of the presence of sperm in NOA patients, it is dependent on the pathologic etiology of azoospermia and may not always be a reliable predictive factor. [22, 23] The various demographic characteristics in each study may explain the differences in these results. Li et al. considered FSH to only reflect the global spermatogenesis function but stated that FSH cannot determine the function of an isolated area in a testis. Micro-TESE is able to retrieve sperm even if the global spermatogenesis function of the testis is very low. This could be the reason why FSH could not precisely predict the sperm retrieval rate of micro-TESE. [23] In our study, we found that the FSH levels did indeed differ greatly in patients with whom sperm was retrieved successfully vs patients where sperm was not retrieved. However, the difference was not significant (see Table 3). In the NOA group, the median FSH level at which sperm was retrieved was found to be 23.17 IU/L versus 32.78 IU/L in men where sperm was not retrieved (P=0.062). Besides, 5.82 and 5.93 were the values of FSH in successful and unsuccessful semen retrieval in the OA patients, respectively.

LH is not commonly used as a predictor of sperm retrieval outcome. In males, LH stimulates Leydig cells to make and release testosterone into the testes and the blood. In contrast to FSH, LH appears to have little role in spermatogenesis outside of inducing gonadal testosterone production. Cissen et al. found that LH was also predictive for successful sperm retrieval. [9] Enatsu et al. demonstrated that older age and non-idiopathic etiology were significantly associated with the probability of successful sperm retrieval. However, they found no significant effects on testicular volume, FSH, LH, or testosterone on sperm retrieval. [20] Our data also found no significance difference of LH value between the two groups in OA and NOA patients (P>0.05)

In our study, varicocele was found in 16 cases (11.8%). Varicoceles were present in 26% of Chinese male patients with infertility. [30] The diagnosis of varicocele by physical examination has a specificity of approximately 70%. In contrast, spectral Doppler analysis has a sensitivity of approximately 97% and a specificity of 94%. [31] Levinger U et al. found that the prevalence of varicocele increases with age. The prevalence of a varicocele increases 10% for each decade of life with the prevalence reaching 75% in the eight decade. [32] Although the relationship between varicocele and male infertility has been demonstrated in many studies, the exact mechanism of the effect of a varicocele on spermatogenesis remains unknown. [30] In our study, there was no significant difference between varicoceles and sperm retrieval outcomes. Scrotal ultrasound has a role in the diagnosis of the etiologies of male infertility. Abdulwahed et al. divided 268 infertile males into an OA versus NOA groups based on histopathology. The authors found, using ultrasonography, that decreased testicular volume and varicoceles (intra- and extra-testicular) were the most common abnormalities in NOA patients. In contrast, epididymitis,
spermatocele and duct ectasia were the most common in OA patients with a sensitivity 87% but only 30% specificity. Therefore, scrotal ultrasound is used to exclude these diagnoses. [17]

Sharath et al. (2013) found that a mean testicular volume is 15 ml (right testis 7.62±4.056 ml, left testis 6.99±3.60 ml) in males with an abnormal semen analysis. [14] There is a distinction between the selection of sperm recovery methods in OA patients (TESA, Percutaneous Biopsy, PESA, MESA) vs NOA patients (TESE, micro TESE). In particular, we found that the median testicular volume was 14.46 ml (right testicular volume 14.32 ml and left testicular volume 14.60 ml) in OA patients compared to 4.68 ml (Right testis 4.67 and left testis 4.69 ml) in NOA patients. According to Moon et al. testicular volume was found to be statistically significant between the OA and NOA patients (mean testicular volume of OA and NOA patients was 11.6 ml and 8.3 ml, respectively, \( P<0.05 \)). [33] Huang et al. showed that a combination of FSH >9.2 mIU/ml and right testis size <15 ml may be used to distinguish NOA patients from OA patients. The positive predictive value for NOA patients was 99.2% and for OA patients was 81.8%. [21] In fact, many studies showed that testicular volume was not reduced in OA patients. Despite the difference in testicular volume between these studies, the majority of studies showed that the mean total testicular volume in infertile males was lower than normal. There was an insignificant difference in total testicular volume between the successful and unsuccessful sperm retrieval groups (5.68 ml versus 4.46 ml in NOA group, \( P=0.138 \); 14.90 ml versus 11.14 ml in OA group, \( P=0.208 \)). Tang WH et al. and Enatsu N et al. found that the testicular volume of the successful sperm retrieval group was higher than that of the unsuccessful sperm retrieval group, however there was no statistical significance between the two groups. [20, 34] Salehi et al. (2017) performed TESE and micro-TESE in 170 NOA patients and found that the sperm recovery rate was 48.8% and that testicular volume was related to the surgical sperm retrieval results (OR,10.5, \( P<0.01 \)). [16] The result was similar to Boitrelle’s study. [18]

In our study, successful sperm retrieval in the OA and NOA groups was 88.3% and 18.4%, respectively (\( P<0.001 \)). In the OA group, sperm production was totally normal, so the sperm retrieval rates via TESA or PESA was very high. Levine et al. assessed the effect of PESA and TESA in the NOA and the OA groups and found that SRR in the NOA group was 47% and OA this rate amounted to 100%. [35]

In fact, it is difficult to predict the outcome of surgical sperm retrieval with only testicular volume or any hormone value as many factors can affect the process of spermatogenesis. Therefore, a combination of these values may prove to be more reliable. However, after multivariate logistic regression analysis, we found no significance difference between the two groups according to the results of semen retrieval. Boitrelle et al. found that there was no significant difference between the two groups (sperm present and no sperm) with age, testosterone, or LH. However, they found that a combination of FSH concentration, inhibin B, and total testicular volume were the best predictors of TESE outcomes (AUC=0.663). [18] Li H et al. analyzed five studies with a total of 1764 patients involving testicular volume and found AUC=0.6389, indicating a low predictive value. [23] Boitrelle et al. developed a formula to predict TESE outcome that included three parameters: total testicular volume, FSH and inhibin B. This formula was shown to be the best predictor of successful TESE (positive likelihood ratio:+3.01). [18] Otherwise, certain studies showed that testicular volume did not affect the sperm retrieval rate for micro-TESE. These studies suggested that
Testicular volume was an influential factor in successful sperm retrieval, as it is correlated with spermatogenesis, however topographical variations in testicular pathology can occur. Consequently testicular volume may not be a good predictive factor for successful sperm retrieval for ICSI. [22, 36]

Ramasamy et al. found that BMI and age had no predictive value for sperm retrieval rates [37, 38] which was similar to the findings in our study. From our research, preoperative variables, namely FSH and testicular volume could not be used as a predictive factor for the success of sperm retrieval in men with non-obstructive azoospermia. Histopathological examination has been the most reliable predictive factor of SRR to date. However, it is contraindicated to perform a testicular biopsy just to predict the SRR of microdissection TESE. [23] We believe that a trial for sperm retrieval should not be denied to any man with azoospermia based solely on the values of the preoperative variables. The patient should be informed, prior to the operation that finding mature cells may not be guaranteed (even in cases with normal FSH and testicular volume) and allowed to make an informed decision on whether to proceed.

In conclusion, in a variety of optimal cut-off values for testis volume and endocrine profiles for men with azoospermia have been reported with controversial results. This study demonstrated that testicular volume and endocrine tests should not be used as predictive factors for sperm retrieval outcomes in infertile males with azoospermia, both in the OA and NOA groups.

**Abbreviations**

ART: assisted reproductive technologies; BMI: Body Mass Index; CDUS: color Doppler ultrasound; FSH: Follicle stimulating hormone, HUECREI: Hue Center for Reproductive Endocrinology and Infertility; ICSI: intracytoplasmic sperm injection; IVF: in-vitro fertilization; LH: Luteinizing hormone; Micro-TESE: Microsurgical testicular sperm extraction; NOA: non-obstructive azoospermia; OA: obstructive azoospermia; PESA: percutaneous epididymal sperm aspiration, SR: sperm retrieval; TESA: Testicular sperm aspiration; TESE: testicular sperm extraction; WHO: World Health Organization.

**References**

1. Aziz N. The importance of semen analysis in the context of azoospermia. Clinics (Sao Paulo). 2013;68:35-8.
2. Jarow JP, Coburn M, Sigman M. Incidence of varicoceles in men with primary and secondary infertility. Urology 1996;47:73-6.
3. World Health Organization. WHO laboratory manual for the examination and processing of human semen. Organization WH, editor. Geneva: World Health Organization 2010.
4. Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, Dohle G, et al. European Association of Urology guidelines on Male Infertility: the 2012 update. Eur Urol 2012;62:324-32.
5. Esteves SC, Miyaoka R, Agarwal A. Sperm retrieval techniques for assisted reproduction. Int Braz J Urol 2011;37:570-83.
6. Matsumoto AM, Bremner WJ. Endocrinology of the hypothalamic-pituitary-testicular axis with particular reference to the hormonal control of spermatogenesis. Baillieres Clin Endocrinol Metab 1987;1:71-87.
7. Wosnitzer MS, Goldstein M. Obstructive azoospermia. Urol Clin North Am 2014;41:83 - 95.
8. Sabanegh E, Agarwal A. Male infertility. In: Wein A, Kavousi L, editors. Campbell – Walsh urology Saunders/Elsevier. 2012:616-47.
9. Cissen M, Meijerink AM, D’Hauwers KW, Meissner A, van der Weide N, Mochtar MH, et al. Prediction model for obtaining spermatozoa with testicular sperm extraction in men with non-obstructive azoospermia. Hum Reprod 2016;31:1934-41.
10. Yang Q, Huang YP, WangHX, Hu K, Wang YY, Huang YR, et al. Follicle-stimulating hormone as a predictor for sperm retrieval rate in patients with nonobstructive azoospermia: a systematic review and meta-analysis. Asian J Androl 2015;17:218-4.
11. Dogra VS, Gottlieb RH, Oka M, Rubens DJ. Sonography of the Scrotum. Radiology 2003;227:18-36.
12. Sakamoto H, Saito K, Oohta M, Inoue K, Ogawa Y, Yoshida H. Testicular volume measurement: comparison of ultrasonography, orchidometry, and water displacement. Urology 2007;69:152-7.
13. Kristo A, Dani E. The correlation between Ultrasound testicular volume and conventional semen parameters in Albanian subfertile males. Open Access Maced J Med Sci 2014;2:464-6.
14. Sharath KC, Najafi Mohsen, Vineeth VS, Malini S.S. Assessment of testicular volume in correlation with Spermiogram of Infertile males in South India. Adv Stud Biol 2013;5:327-35.
15. Setchell BP, Brooks DE. Anatomy, vasculature, innervation and fluids of the male reproductive tract. In: Jimmy D. Neill, editor. Knobil and Neill's Physiology of Reproduction Third ed: Elsevier; 2006.
16. Salehi P, Derakhshan-Horeh M, Nadeali Z, Hosseinzadeh M, Sadeghi E, Izadpanahi MH, et al. Factors influencing sperm retrieval following testicular sperm extraction in nonobstructive azoospermia patients. Clin Exp Reprod Med 2017;44:22-7.
17. Abdulwahed SR, Mohamed EE, Taha EA, Saleh MA, Abdelsalam YM, ElGanainy EO. Sensitivity and specificity of ultrasonography in predicting etiology of azoospermia. Urology 2013;81:967-71.
18. Boitrelle F, Robin G, Marcelli F, Albert M, Leyroy-Martin B, Dewaily D, et al. A predictive score for testicular sperm extraction quality and surgical ICSI outcome in non-obstructive azoospermia: a retrospective study. Hum Reprod 2011;26:3215-21.
19. Chen SC, Hsieh JT, Ju HJ, Chang HC. Appropriate cut-off value for follicle-stimulating hormone in azoospermia to predict spermatogenesis. Reprod Biol Endocrinol 2010;8:108.
20. Enatsu N, Miyake H, Chiba K, Fujisawa M. Predictive factors of successful sperm retrieval on microdissection testicular sperm extraction in Japanese men. Reprod Med Biol 2015;15:29-33.
21. Huang IS, Huang WJ, Kin AT. Distinguish non-obstructive azoospermia from obstructive azoospermia in Taiwanese patients by hormone profile and testis size. J Chin Med Assoc 2018;81:531-5.
22. Ziaee SA, Ezzatnegad M, Nowroozi M, Jamshidian H, Abdi H, Hosseini Moghaddam SM. Prediction of Successful Sperm Retrieval in Patients with Nonobstructive Azoospermia. Urol J 2006;3:92-6.
23. Li H, Chen LP, Yang J, Li MC, Chen RB, Lan RZ, et al. Predictive value of FSH, testicular volume, and histopathological findings for the sperm retrieval rate of microdissection TESE in nonobstructive azoospermidia: a meta-analysis. Asian J Androl 2018;20:30-6.
24. Schoor RA, Elhanbly S, Niederberger CS, Ross LS. The role of testicular biopsy in the modern management of male infertility. J Urol 2012;167:197 - 200.
25. Sarteschi LM. Lo studio del varicocele con eco-color-Doppler. G Ital Ultrasonologia 1993;4:43-9.
26. Ramasamy R, Yagan N, Schlegel PN. Structural and functional changes to the testis after conventional versus microdissection testicular sperm extraction. Urology 2005;65:1190-4.
27. Ishikawa T. Surgical recovery of sperm in non-obstructive azoospermidia. Asian J Androl 2012;14:109-15.
28. Layman LC, Porto AL, Xie J, Da Motta LA, Weiser W, Sluss PM. FSHβ Gene Mutations in a Female with Partial Breast Development and a Male Sibling with Normal Puberty and Azoospermidia. J Clin Endocrinol Metab 2002;87:3702-7.
29. Layman LC, McDonough PG. Mutations of follicle stimulating hormone-beta and its receptor in human and mouse: genotype/phenotype. Mol Cell Endocrinol 2000;161:9-17.
30. Zhang Y, Ma T, Su Z, Ye M, Tian H, Li J, et al. Varicoceleffect semen quality of infertile men in Southern China: A cross-sectional study of 5447 cases. Medicine (Baltimore). 2017;96:e7707.
31. Trum JW, Gubler FM, Laan R, van der Veen F. The value of palpation varicoscreen contact thermography and colour Doppler ultrasound in the diagnosis of varicocele. Hum Reprod 1996;11:1232-5.
32. Levinger U, Gornish M, Gat Y, Bachar GN. Is varicocele prevalence increasing with age? Andrologia 2007;39:77-80.
33. Moon MH, Kim SH, Cho JY, Seo JT, Chun YK. Scrotal US for evaluation of infertile men with azoospermidia. Radiology 2006;239:168-73.
34. Tang WH, Jiang H, Ma LL, Hong K, Zhao LM, Mao JM, et al. Correlation of testicular volume and reproductive hormone level with the result of testicular sperm aspiration in non-obstructive azoospermidia patients. Zhonghua nan ke xue = National Journal of Andrology 2012;18:48-51.
35. Levine LA, Dimitriou RJ, Fakouri B. Testicular and epididymal percutaneous sperm aspiration in men with either obstructive or non-obstructive azoospermidia. Urology 2003;62:328-32.
36. Bryson CF, Ramasamy R, Sheehan M, Palermo GD, Rosenwaks Z, Schlegel PN. Severe testicular atrophy does not affect the success of microdissection testicular sperm extraction. J Urol 2014;191:175-8.
37. Ramasamy R, Bryson C, Reifsnyder JE, Neri Q, Palermo GD, Schlegel PN. Overweight men with nonobstructive azoospermidia have worse pregnancy outcomes after microdissection testicular sperm extraction. Fertil Steril 2013;99:372-6.
38. Ramasamy R, Trivedi NN, Reifsnyder JE, Palermo GD, Rosenwaks Z, Schlegel PN. Age does not adversely affect sperm retrieval in men undergoing microdissection testicular sperm extraction. Fertil
Tables

Table 1. General characteristics of the men in obstructive azoospermia (OA) and non-obstructive azoospermia (NOA) groups

| Variables                        | Total (n=136) | OA (n=60) | Non OA (n=76) | p-value |
|----------------------------------|---------------|-----------|---------------|---------|
| Age (years)                      | 35.54±8.36    | 37.38±10.63 | 34.08±5.65    | 0.485   |
| <35                              | 78 (57.4)     | 32 (53.3)  | 46 (60.5)     |         |
| ≥35                              | 58 (42.6)     | 28 (46.7)  | 30 (39.5)     |         |
| Geography                        |               |           |               |         |
| Urban                            | 56 (41.2)     | 26 (43.3)  | 30 (39.5)     | 0.726   |
| Rural                            | 80 (58.8)     | 34 (56.7)  | 46 (60.5)     |         |
| Education                        |               |           |               |         |
| School grade                     | 83 (61.0)     | 36 (60.0)  | 47 (61.8)     | 0.506   |
| University grade                 | 53 (39.0)     | 24 (40.0)  | 29 (38.2)     |         |
| Occupation                       |               |           |               |         |
| Office work                      | 47 (34.6)     | 20 (33.3)  | 27 (35.5)     | 0.857   |
| Manual work                      | 89 (65.4)     | 40 (66.7)  | 49 (64.5)     |         |
| Infertility type                 |               |           |               |         |
| Primary                          | 125 (91.9)    | 53 (88.3)  | 72 (94.7)     | 0.213   |
| Secondary                        | 11 (8.1)      | 7 (11.7)   | 4 (5.3)       |         |
| Infertility duration (years)     |               |           |               |         |
| <3                              | 56 (41.2)     | 23 (38.3)  | 33 (43.4)     | 0.601   |
| ≥3                              | 80 (58.8)     | 37 (61.7)  | 43 (56.6)     |         |
| Mump                             |               |           |               |         |
| Yes                              | 46 (33.8)     | 15 (25.0)  | 31 (40.8)     | 0.068   |
| No                               | 90 (66.2)     | 45 (75.0)  | 45 (59.2)     |         |
| History surgery                  |               |           |               |         |
| Yes                              | 20 (14.7)     | 12 (20.0)  | 8 (10.5)      | 0.147   |
| No                               | 116 (85.3)    | 48 (80.0)  | 68 (89.5)     |         |
| Testicular density n (%)         |               |           |               | <0.01   |
| Normal                           | 77 (56.6)     | 45 (75.0)  | 32 (42.1)     |         |
| Abnormal                         | 59 (43.4)     | 15 (25.0)  | 44 (57.9)     |         |
| Varicocele                       |               |           |               |         |
| Yes                              | 16 (11.8)     | 4 (6.7)    | 12 (15.8)     | 0.116   |
| No                               | 120 (88.2)    | 56 (93.3)  | 64 (84.2)     |         |
OA: obstructive azoospermia; NOA: non-obstructive azoospermia

| Variables                             | OA                       | NOA                      |
|---------------------------------------|--------------------------|--------------------------|
|                                       | Sperm (+) | Sperm (-) | p-value | Sperm (+) | Sperm (-) | p-value | Sperm (+) | Sperm (-) | p-value |
| Age (years)                           |            |            |         |            |            |         |            |            |         |
| <35                                   | 27 (50.9)  | 5 (71.4)  | 0.432   | 7 (50.0)  | 39 (62.9) | 0.384   |
| ≥35                                   | 26 (49.1)  | 2 (28.6)  |          | 7 (50.0)  | 23 (37.1) |          |
| Geography                             |            |            |         |            |            |         |            |            |         |
| Urban                                 | 25 (47.2)  | 1 (14.3)  | 0.126   | 8 (57.1)  | 22 (35.5) | 0.225   |
| Rural                                 | 28 (52.8)  | 6 (85.7)  |          | 6 (42.9)  | 40 (64.5) |          |
| Education                             |            |            |         |            |            |         |            |            |         |
| School grade                          | 31 (58.5)  | 5 (71.4)  | 0.732   | 7 (50.0)  | 40 (64.5) | 0.368   |
| University grade                      | 22 (41.5)  | 2 (28.6)  |          | 7 (50.0)  | 22 (35.5) |          |
| Occupation                             |            |            |         |            |            |         |            |            |         |
| Office work                           | 19 (35.8)  | 1 (14.3)  | 0.407   | 8 (57.1)  | 19 (30.6) | 0.072   |
| Manual work                           | 34 (64.2)  | 6 (85.7)  |          | 6 (42.9)  | 43 (69.4) |          |
| Infertility type                       |            |            |         |            |            |         |            |            |         |
| Primary                               | 47 (88.7)  | 6 (85.7)  | 1.000   | 11 (78.6) | 61 (98.4) | 0.018   |
| Secondary                             | 6 (11.3)   | 1 (14.3)  |          | 3 (21.4)  | 1 (1.6)   |          |
| Infertility duration (years)           |            |            |         |            |            |         |            |            |         |
| < 3                                   | 20 (37.7)  | 3 (42.9)  | 1.000   | 7 (50.0)  | 26 (41.9) | 0.766   |
| ≥ 3                                   | 33 (62.3)  | 4 (57.1)  |          | 7 (50.0)  | 36 (58.1) |          |
| History of Mump                       |            |            |         |            |            |         |            |            |         |
| Yes                                   | 15 (28.3)  | 0 (0.0)   | 0.176   | 7 (50.0)  | 24 (38.7) | 0.550   |
| No                                    | 38 (71.7)  | 7 (100.0) |          | 7 (50.0)  | 38 (61.3) |          |
| History of urogenital surgery         |            |            |         |            |            |         |            |            |         |
| Yes                                   | 12 (22.6)  | 0 (0.0)   | 0.326   | 2 (14.3)  | 6 (9.7)   | 0.635   |
| No                                    | 41 (77.4)  | 7 (100.0) |          | 12 (85.7) | 56 (90.3) |          |
| Testicular density n (%)              |            |            |         |            |            |         |            |            |         |
| Normal                                | 39 (73.6)  | 6 (85.7)  | 0.767   | 10 (71.4) | 22 (35.5) | 0.039   |
| Abnormal                              | 14 (26.4)  | 1 (14.3)  |          | 4 (28.6)  | 40 (64.5) |          |
| Varicocele                            |            |            |         |            |            |         |            |            |         |
| Yes                                   | 3 (5.7)    | 1 (14.3)  | 0.399   | 2 (14.3)  | 10 (16.1) | 1.000   |
| No                                    | 50 (94.3)  | 6 (85.7)  |          | 12 (85.7) | 52 (83.9) |          |
OA: obstructive azoospermia; NOA: non-obstructive azoospermia; FSH: follicle-stimulating hormone; LH: luteinizing hormone.

Table 3. Predictive markers of results of sperm retrievals in OA and NOA groups

| Factors          | SR in NOA group | SR in OA group |
|------------------|-----------------|----------------|
|                  | Total           | Successful     | Unsuccessful  | p-value | Total           | Successful     | Unsuccessful  | p-value |
| LH (mIU/ml)      | 15.06±7.62      | 12.08±8.61     | 15.73±7.29    | 0.106    | 5.36±3.133      | 5.56±3.08      | 3.79±3.34     | 0.162   |
| FSH (mIU/ml)     | 31.01±16.75     | 23.17±16.41    | 32.78±16.44   | 0.062    | 5.83±3.81       | 5.82±3.68      | 5.93±5.05     | 0.943   |
| Testosterone     | 2.95±1.65       | 2.98±1.10      | 2.94±1.76     | 0.914    | 4.53±2.14       | 4.42±2.13      | 5.36±2.21     | 0.277   |
| Right volume     | 4.67±2.99       | 5.35±1.96      | 4.52±3.17     | 0.222    | 14.32±7.21      | 14.77±7.29     | 10.93±5.94    | 0.188   |
| Left volume      | 4.69±3.24       | 6.01±4.52      | 4.39±2.84     | 0.092    | 14.60±8.02      | 15.03±8.19     | 11.36±6.18    | 0.259   |
| Mean volume      | 4.68±2.78       | 5.68±2.37      | 4.46±2.83     | 0.138    | 14.46±7.36      | 14.90±7.47     | 11.14±5.94    | 0.208   |

SR: sperm retrieval; OA: obstructive azoospermia; NOA: non-obstructive azoospermia; FSH: follicle-stimulating hormone; LH: luteinizing hormone

Declarations

This work was approved by the Ethics Committee of Hue University of Medicine and Pharmacy, Vietnam. All participants were explained and agreed with written consent form.

Competing interests: The authors have no competing financial or other interests to declare in relation to this manuscript.