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# Endocrine Tests and/or Testicular Volume are Not Predictive of Successful Sperm Retrieval by Conventional Multiple Testicular Sperm Extraction in Nonobstructive Azoospermia

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## ABSTRACT

**Objectives:** This study aimed to determine the role of presurgical markers in the prediction of sperm retrieval by conventional Multiple Testicular Sperm Extraction in infertile Vietnamese men with nonobstructive azoospermia (NOA).

**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 conventional multiple testicular sperm extraction in up to three locations, and procedures stopped as soon as sperm were detected. Factors were analyzed to determine the prediction of the likelihood of successful sperm retrieval, in men with NOA.

**Results:** The overall success rate of sperm retrieval in men with azoospermia was 49.3%, but it was only 18.4% in NOA group. The difference in testicular volume between men with successful sperm retrieval and unsuccessful sperm retrieval was not statistically significant in NOA group ($5.68 \pm 2.37$ vs. $4.46 \pm 2.83$, $p = 0.138$). The differences in the endocrine tests between the two groups were also not significant in terms of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone ($p > 0.05$). Multivariable analysis of predictive factors of sperm retrieval in NOA groups found no significant difference, except testicular density ($p = 0.015$).

**Conclusions:** In infertile men with NOA, neither an endocrine test nor testicular volume should be used for predicting the results of surgical sperm retrieval by conventional multiple testicular sperm extraction.

***Keywords***: Testicular Volume; Follicular-Stimulating Hormone; Sperm Retrieval; Azoospermia.

## Tóm tắt
## (ABSTRACT IN VIETNAMESE)

**Mục tiêu:** Nghiên cứu này nhằm xác định vai trò tiên lượng của các chỉ dấu trước phẫu thuật trong phương pháp trích mô tinh hoàn đa điểm truyền thống ở các trường hợp vô tinh không do tắc nghẽn tại Việt Nam.

**Chọn bệnh và phương pháp nghiên cứu:** phân tích mô tả hồi cứu 136 nam giới vô tinh, trong thời gian từ tháng 8 năm 2014 đến tháng 7 năm 2018. Những bệnh nhân này được chỉ định phẫu thuật từng bước qua các kỹ thuật chọc hút tinh trùng từ mào tinh, chọc hút tinh trùng từ tinh hoàn và phẫu thuật trích mô tinh hoàn tìm tinh trùng tại tối đa 3 vị trí và sẽ dừng kỹ thuật bất cứ thời điểm nào phát hiện có tinh trùng. Phân tích các yếu tố để dự báo khả năng có thể tìm thấy tinh trùng.

**Kết quả:** Tỷ lệ thành công chung của phẫu thuật trích tinh trùng từ các trường hợp vô tinh là 49.3%, nhưng tỷ lệ này chỉ là 18.4% ở nhóm vô tinh không do tắc nghẽn. Sự khác biệt về thể tích tinh hoàn là không có ý nghĩa thống kê giữa hai nhóm vô tinh không do tắc nghẽn có và không tìm thấy tinh trùng ($5.68 \pm 2.37$ vs. $4.46 \pm 2.83$, $p = 0.138$). Sự khác biệt về nội tiết LH, FSH và testosterone giữa hai nhóm cũng không có ý nghĩa ($p > 0.05$). Phân tích đa biến các yếu liên quan đến khả năng tìm thấy tinh trùng ở nhóm vô tinh không do tắc nghẽn đã không thấy sự khác biệt có ý nghĩa, ngoại trừ mật độ tinh hoàn ($p = 0.015$).

**Kết luận:** Ở nam giới vô tinh không do tắc nghẽn, cả xét nghiệm nội tiết và thể tích tinh hoàn đều không có giá trị dự báo kết quả phẫu thuật trích tinh trùng bằng kỹ thuật trích mô tinh hoàn đa điểm truyền thống.

**Từ khoá:** thể tích tinh hoàn, follicular-stimulating hormone, phẫu thuật trích tinh trùng, vô tinh


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## INTRODUCTION

Azoospermia is one of the causes of infertility. It is reported in approximately 1% of males and 10%–15% of infertile males (Aziz et al., 2013; Jarow et al., 1996). It includes nonobstructive azoospermia (NOA) and obstructive azoospermia (OA) (WHO, 2010). OA is less common than NOA and occurs in 15%–20% of men with azoospermia (Jungwirth et al., 2012). 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 (Esteves et al., 2011).

In fact, abnormal spermatogenesis may be caused by any factor related to the hypothalamic–pituitary–testicular axis, including the effects of follicle-stimulating hormone (FSH), luteinizing hormone (LH), or testosterone on the function of the testis (Matsumoto and Bremner, 1987). 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 have a lower chance of achieving successful sperm extraction (Wosnitzer and Goldstein, 2014). With assisted reproductive technologies (ARTs), 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) in conjunction with intracytoplasmic sperm injection (ICSI). Currently, microsurgical TESE (microTESE) is superior to conventional TESE and is the gold standard for testicular sperm recovery in NOA (Schwarzer et al., 2015). Overall successful sperm retrieval ranged from 16.7%–45% using conventional TESE, compared to 42.9%–63% in the microTESE group (Deruyver et al., 2013). Unfortunately, microTESE cannot be applied to all cases with NOA because of skillfulness requirement, special equipment, and prolonged procedure (Li et al., 2018; Moon et al., 2006). Furthermore, mature sperm are not found in certain cases and these invasive procedures may be unsuccessful. Therefore, it is important to have certain noninvasive testing that is able to predict spermatogenesis. Currently, an accurate means of predicting spermatogenesis and successful sperm retrieval do not exist.

Testicular function can be assessed by clinical examination, hormonal tests, semen analysis, scrotal ultrasound, or biopsy of the testis (Sabanegh and Agarwal, 2012). Based on the activities on the hypothalamic–pituitary–testicular axis, serum FSH and LH have been reported as important preoperative markers reflecting testicular function (Matsumoto and Bremner, 1987). Serum FSH and LH concentration is inversely related to the sperm retrieval rate (Cissen et al., 2016). 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 order to confirm the diagnostic value of other noninvasive parameters (Yang et al., 2015).

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 (Dogra et al., 2003). 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 formula for calculation of the testicular volume (Sakamoto et al., 2007). Testicular volume has been noted to have a direct correlation with semen profiles (Kristo and Dani, 2014; Sharath et al., 2013) as well as the production ability of sperm in males (Setchell and Brooks, 2006).

It is reported that the recovery of spermatozoa is successful in approximately 50% of men with azoospermia, and therefore, the prediction of the success rate of sperm retrieval is necessary before attempted surgery (Salehi et al., 2017). By using the current popular method of sperm retrieval, microTESE, no single clinical finding or test that is able to predict successful sperm retrieval precisely has been found. Furthermore, a variety of optimal cutoff values for testes volume and endocrine profiles for infertile males have been reported (Boitrelle et al., 2011; Chen et al., 2010; Cissen et al., 2016; Enatsu et al., 2015; Flannigan et al., 2017; Huang et al., 2018; Ziaee et al., 2006). This is possibly due to the effects of the study population and ethnicity (Li et al., 2018). Although the diagnosis of OA and NOA is based on histological examination, initial categorization between the two groups before surgery can be suggested by presurgical markers, including FSH and testicular measurement. This approach is practical and useful for clinicians in predicting prognosis and counseling (Schoor et al., 2002). This study aimed to determine the role of presurgical markers in the prediction of the likelihood of successful sperm retrieval in infertile men with azoospermia in a Vietnamese population.

## PATIENTS AND METHODS

### Patient selection and evaluation

This was a retrospective cohort study that included 136 infertile males with azoospermia on semen analysis based on the World Health Organization (WHO) 2010 standard criteria. 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 ($3{,}000 \times g$ for 15 minutes) (WHO, 2010). The testicular biopsy was not performed for all cases with azoospermia, therefore in this study, the diagnosis of NOA and OA was not established upon histological examination but the classification was based on presurgical markers. According to Schoor RA et al. azoospermia patients with FSH ≥ 7.6 mIU/mL with testicular long axis ≤ 46 mm were categorized into the NOA group. The remainder of the patients belonged to the OA group (Schoor et al., 2002). All patients routinely underwent the evaluation of chromosome karyotyping and Y chromosome microdeletion. Patients with acute systemic diseases, acute urinary tract infection, hepatic function disorders, malignant diseases, retrograde ejaculation, hypogonadotropic hypogonadism, and Y chromosome microdeletions, known as obstructive azoospermia (e.g., postvasectomy) were excluded. All patients' general characteristics including age, geography, education, occupation, clinical history, physical examination, infertility type, infertility duration, history of mumps, and history of surgery on reproductive urinary tract were recorded. Testicular density was determined by palpable examination to distinguish the testis as normal or soft (abnormal).

### Scrotal ultrasound technique

Testicular ultrasound was performed to evaluate testicular echogenicity and homogeneity in two-dimensional (2D) ultrasound and the presence of varicocele was assessed on 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 longitudinal 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 any 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 (Sarteschi, 1993). 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, United States). The FSH's interassay coefficient of variation (interassay 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 stepwise surgical sperm retrieval via three techniques: PESA, TESA, and then multiple TESE consecutively, in maximum three locations and the procedure was stopped at any step if sperm was found. Each procedure was carried out on the right and then the left testis in turn. The scrotum was cleaned with antiseptic before being washed by saline to eliminate 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 three 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 ×300 magnification to detect spermatozoa. The surgical procedure was stopped once the lab staff found sperm 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-squared test, and an independent sample T-test. If the data were not normally distributed, then the Wilcoxon rank–sum test

Table 1. General characteristics of the men in obstructive azoospermia (OA) and nonobstructive 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 | |
| <35 | 78 (57.4) | 32 (53.3) | 46 (60.5) | 0.485 |
| ≥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) | |
| History of mumps | | | | |
| 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 (%) | | | | |
| Normal | 77 (56.6) | 45 (75.0) | 32 (42.1) | <0.01 |
| Abnormal | 59 (43.4) | 15 (25.0) | 44 (57.9) | |

NOA: nonobstructive azoospermia; OA: obstructive azoospermia.

(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

Based on presurgical markers including FSH and testicular measurement, 136 infertile men with azoospermia were categorized as 60 with OA and 76 with NOA cases. Surgical sperm retrieval was, in general, successful in 67 of 136 patients (49.3%), in which, the success rate of OA and NOA group was 88.3% and 18.4%, respectively. 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 is no significant difference between the two groups regarding the patient's age, geography, education, occupation, infertility type, infertility duration, history of surgery, and/or mumps. There is significant difference in testicular density between the two 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 is no significant difference in the clinical characteristics between the two groups. Besides, the abnormal testicular density, determined by palpation, is significantly higher in unsuccessful sperm retrieval group than that of successful group (64.55 vs. 28.6%, $p = 0.039$). Other characteristics were not significant between the two groups.

The comparisons of testicular volume and endocrine tests between the two groups according to the sperm retrieval outcomes (successful and unsuccessful) are shown in Table 3 by bivariable analysis. In both, the NOA and OA groups, the difference in testicular volume between men with successful sperm retrieval and unsuccessful sperm retrieval 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 ($p > 0.05$). Similar results were also recorded by multivariable analysis of predictive factors of sperm retrieval in both OA and NOA groups with no significant difference, except testicular

Table 2. Clinical characteristics of successful and unsuccessful sperm retrieval in obstructive azoospermia (OA) and nonobstructive azoospermia (NOA) groups.

| Variables | OA (n = 60) | | | NOA (n = 76) | | |
|---|---|---|---|---|---|---|
| Sperm retrieval | Sperm (+) n = 53 (88.3%) | Sperm (−) n = 7 (11.7%) | p value | Sperm (+) n = 14 (18.4%) | Sperm (−) n = 62 (81.6%) | 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) | |

FSH: follicle-stimulating hormone; LH: luteinizing hormone; NOA: nonobstructive azoospermia; OA: obstructive azoospermia.

Table 3. Bivariable analysis of predictive factors of sperm retrieval in obstructive azoospermia (OA) and nonobstructive azoospermia (NOA) groups.

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

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

Table 4. Multivariable analysis of predictive factors of sperm retrieval in obstructive azoospermia (OA) and nonobstructive azoospermia (NOA) groups.

| | SR in OA group (n = 60) | | | SR in NOA group (n = 76) | | |
|---|---|---|---|---|---|---|
| **Factors** | **Coefficient** | **95% CI** | **p value** | **Coefficient** | **95% CI** | **p value** |
| Endocrine tests | | | | | | |
| LH (mIU/mL) | 0.89 | 0.16–1.63 | 0.017 | 0.05 | −0.11–0.20 | 0.568 |
| FSH (mIU/mL) | −0.39 | −0.80–0.02 | 0.065 | −0.04 | −0.11–0.04 | 0.341 |
| Testosterone (nmol/L) | −0.79 | −1.66–0.09 | 0.078 | −0.48 | −1.06–0.10 | 0.103 |
| Testicular ultrasound | | | | | | |
| Right volume | 0.14 | −0.14–0.42 | 0.312 | −0.003 | −0.33–0.32 | 0.987 |
| Left volume | −0.03 | −0.25–0.20 | 0.828 | 0.18 | −0.06–0.43 | 0.133 |
| Testicular density | 0.87 | −1.61–3.35 | 0.490 | −2.01 | −3.62–0.40 | 0.015 |

CI: confidence interval; FSH: follicle-stimulating hormone; LH: luteinizing hormone; NOA: nonobstructive azoospermia; OA: obstructive azoospermia; SR: sperm retrieval.



density, which is significantly different in NOA group ($p = 0.015$), as shown in Table 4.

## DISCUSSION

This study investigated the predictive value of clinical examination, ultrasound parameters, and hormone levels for successful sperm retrieval in azoospermia, focused on NOA patients. Although histological biopsy is the gold standard to distinguish OA and NOA groups, various publications have used testicular volume and levels of FSH as an indicative marker (Flannigan et al., 2017; Huang et al., 2018; Schoor et al., 2002). However, this approach leaves open the possibility of misclassification of some patients. Our retrospective study collected data from the previous 5 years of clinical practice of a University hospital. Because of the limitation of absence of histology from all samples, the classification of OA and NOA should be clarified as probability/likelihood before surgery rather than definitely diagnosis. Schoor et al. have shown that an FSH of less than 7.6 mIU/mL and testicular long axis greater than 4.6 cm predicts OA in 96% of cases, and FSH of more than 7.6 mIU/mL with a testicular long axis of less than 4.6 cm predicts NOA in 89% of cases (Schoor et al., 2002). Flannigan and Schlegel have given a flow chart to depict surgical management of azoospermia patients based mainly upon FSH and testicular volume. FSH with 7.5 IU/L thresholds and testicular volume were two very important features to differentiate OA or NOA (Flannigan et al., 2017). A recent publication in 2018 comparing the ability to diagnose OA or NOA by FSH and testicular volume with the result of biopsy have indicated that an FSH level >9.2 mIU/mL and testis size <15 mL will be predictive and can avoid unnecessary testicular biopsies (Huang et al., 2018). The optimal cutoff value for FSH in this study was 9.2 mIU/mL, which is higher compared to previous studies. This could be explained by different ethnicity. The difference of cutoff values between various studies emphasizes the necessity of adjusting thresholds values based on the population to improve the diagnostic accuracy. In our study, designed as a retrospective method, based on the clinical practice of the center, patients underwent stepwise surgical sperm retrieval via PESA, TESA then multiple TESE in up to three locations, and procedures were stopped as soon as sperm were detected. The biopsy was performed only in cases without sperm after the last step (TESE). Based on the criteria for OA/NOA classification of Schoor et al. FSH and testicular volume to differentiate between the two groups. Biopsy was not indicated routinely for all patients to distinguish OA or NOA due to the limited clinical benefits and ethical issues.

The combined sperm retrieval rate in this study was 49.3%, but it was only 18.4% in NOA group although we did multiple TESE, up to three locations before abandoning the procedures. This sperm retrieval rate was much lower than previous study reported by Salehi et al. (Salehi et al., 2017). Ramasamy et al. reported sperm retrieval rates of 32% with conventional TESE and 57% with microsurgical TESE (MicroTESE) (Ramasamy et al., 2005). Similarly, Ishikawa et al. showed the sperm retrieval rate of 42% when they performed microTESE for NOA patients (Ishikawa, 2012). The various results between these studies could be partially explained by low effectiveness of applied conventional TESE, especially compared to microTESE. In terms of the OA, the 88% retrieval rate was lower than expected (Schoor et al., 2002). Our use of a clinically convenient algorithm based on serum FSH and testicular volume for diagnosis, rather than histology, may have resulted in patients with NOA being misclassified as OA, which may then explain the failure of sperm recovery in 7 of 60 cases.

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 (Layman and McDonough, 2000; Layman et al., 2002). Serum FSH was reported to predict the existence of sperm, which could be retrieved by conventional TESE (Ishikawa, 2012). Elevated plasma levels of FSH above 19.4 mIU/mL suggest no spermatogenesis and hence predicts an unsuccessful sperm retrieval (Chen et al., 2010). However, recent studies showed that FSH has a low predictive value for the outcome of sperm retrieval. Although serum FSH is a 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 (Li et al., 2018; Ziaee et al., 2006). 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. MicroTESE 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 microTESE (Li et al., 2018). In our study, by conventional multiple dissections of the testicular extraction, although we found that the FSH levels did indeed differ greatly in NOA patients whose sperm was retrieved successfully compared to patients without sperm retrieved (23.17 ± 16.41 vs. 32.78 ± 16.44 mIU/mL). However, the difference was not significant (see Table 3).

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 (Cissen et al., 2016). Enatsu et al. demonstrated that older age and nonidiopathic 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 (Enatsu et al., 2015). Our data also found no significance difference of LH value between the two groups in OA and NOA patients ($p > 0.05$).

Sharath et al. (2013) found that a mean of total testicular volume was 15 mL (right testis 7.62 ± 4.056 mL, left testis 6.99 ± 3.60 mL) in males with an abnormal semen analysis (Sharath et al., 2013). According to Moon et al. testicular volume was found to be significantly statistically different between the OA and NOA patients (mean testicular volume of OA and NOA patients was 11.6 mL and 8.3 mL, respectively, $p < 0.05$) (Moon et al., 2006). 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% (Huang et al., 2018). 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. Our data revealed 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 mL and left testis 4.69 mL) in NOA patients. There was a nonsignificant difference in total testicular volume between the successful and unsuccessful sperm retrieval groups (5.68 mL vs. 4.46 mL in NOA group, $p = 0.138$; 14.90 mL vs. 11.14 mL in OA group, $p = 0.208$). Similarly, Tang et al. and Enatsu 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 (Enatsu et al., 2015; Tang et al., 2012). Inversely, Salehi et al. performed TESE and microTESE in 170 NOA patients and found that testicular volume was related to the surgical sperm retrieval results (odds ratio [OR]: 10.5; $p < 0.01$) (Salehi et al., 2017). Their result was similar to Boitrelle's study (Boitrelle et al., 2011). Higher testicular volume was also predictive for sperm retrieval according to a retrospective analysis in 2018 (Gnessi et al., 2018). However, more recent studies showed that testicular volume did not affect the sperm retrieval rate for microTESE. 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 (Bryson et al., 2014; Ziaee et al., 2006).

In fact, it is difficult to predict the outcome of surgical sperm retrieval with only testicular volume or any hormone value as factors that can affect the process of spermatogenesis. Therefore, a combination of these values may prove to be more reliable. However, after bivariable or multivariable logistic regression analysis, we found no significant difference between the two groups according to the results of sperm retrieval. Previously, 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) (Boitrelle et al., 2011). Other published studies also reported similar conclusions. Boitrelle et al. also found that there was no significant difference between the two groups (sperm present and no sperm) with age, testosterone, or LH. However, they also found that a combination of FSH concentration, inhibin B, and total testicular volume were the best predictors of TESE outcomes (AUC = 0.663) (Boitrelle et al., 2011). Li et al. analyzed five studies with a total of 1,764 patients involving testicular volume and found AUC = 0.6389, indicating a low predictive value (Li et al., 2018). Besides, the previous studies found that body mass index (BMI) and age had no predictive value for sperm retrieval rates (Ramasamy et al., 2013; Ramasamy et al., 2014), which was similar to the findings in our study.

From our research, preoperative markers, namely FSH and testicular volume could not be used as a predictive factor for the success of sperm retrieval in men with NOA. 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 (Li et al., 2018). In the present study, there was no histological data for all patients because the biopsy was performed only in cases without sperm after the last step—TESE, because of practical aspects. Not having complete data for all cases was a challenge in analyzing results and should be considered as a limitation. 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 predictors. The patient should be informed, prior to the operation that finding mature cells is not guaranteed (even in cases with normal FSH and testicular volume) and allowed to make an informed decision on whether to proceed.

In conclusion, a variety of optimal cutoff 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 by conventional multiple testicular sperm extraction in infertile males with NOA.

**Abbreviations:** ART: Assisted reproductive technologies; BMI: Body mass index; FSH: follicle-stimulating hormone, HUECREI: Hue Center for Reproductive Endocrinology and Infertility; ICSI: intracytoplasmic sperm injection; IVF: in-vitro fertilization; LH: Luteinizing hormone; MicroTESE: 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.

## DECLARATION

### Ethics approval and consent to participate

We, all coauthors, confirm that this work was approved by the Hue University of Medicine and Pharmacy Ethics Committee. All patients agreed to participate with written consent forms.

**Consent for publication:** All authors consent to publication.

**Availability of data and material:** The dataset used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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## AUTHORS' CONTRIBUTIONS

L.M.T, N.T.T.T, N.D.N, T.T.N.Q, N.V.Q.H participated in the study, design, data collection, data analysis, manuscript drafting, and critical discussion. All authors were involved in drafting the work or revising it critically for the important intellectual content of the final manuscript.

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