# TESTICULAR HISTOPATHOLOGY AND ASSOCIATED FACTORS IN MEN WITH NON-OBSTRUCTIVE AZOOSPERMIA

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*The testicular histopathological characteristic in azoospermic men can significantly help in the diagnosis, prognosis and treatment of infertile patients. This study assessed the pathology specimens of 88 azoospermic infertile men seen at Hanoi Medical University Hospital. The most common histopathological pattern was Sertoli cell-only syndrome (40.9%), followed by hypospermatogenesis (29.5%) and Germ cell maturation arrest (18.1%). There were significant differences in the mean testicular volume and serum concentrations of LH and FSH among patients with different histopathology patterns. Analysis using multinomial logistic regression model showed only FSH was significantly associated with the severity of histopathological pattern. Each unit increase in the level of FSH was associated with a 7.1% increase in the odds of a more severe histopathology (95%CI: 1.01 - 1.12).*

**Keywords: Testicular histopathology, azoospermia, infertile men, NOA.**

## I. INTRODUCTION

Infertility is defined as the inability for a couple to conceive after 12 months of regular, unprotected sexual intercourse. Research reported that the men contribute to nearly 50% of all cases[1] and approximately 5%-10% of infertile men are azoospermic (no sperm present in the ejaculate).[2] Data from Hanoi Medical University Hospital showed that azoospermia affected 20.8% of patients who sought consultation for male infertility at the Department of Andrology and Sexual Medicine.[3] Among the etiology of azoospermia, non-obstructive azoospermia (NOA) is the most severe form due to the failure of spermatogenesis and in the past, the only option for fathering a child is donor sperm. However, with the advances of assisted reproductive technique (ART) and microsurgery, the use of micro testicular sperm extraction (microTESE) followed by intracytoplasmic injection (ICSI) has brought the opportunity for these patients to have their own children.

Several factors have been proposed to be the predictors for successful sperm retrieval in men with NOA such as testicular dimensions, follicle-stimulating hormone (FSH), testosterone and testicular histopathology.[4] Although testicular histopathology does not provide the definite cause of infertility, it reveals the impairment of spermatogenesis and the heterogeinity of the testis parenchyma. This information has been demonstrated to provide a significant prognostic value for the sperm retrieval rate in microTESE procedures in previous studies.[5–7] Therefore, we conduct this study to describe the testicular histopathology of NOA men and assess factors associated with each histopathological pattern.

## II. MATERIALS AND METHODS

### 1. Study subjects

Between January 2014 and September 2022, azoospermic infertile men who were

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referred to the Department of Andrology and Sexual Medicine at Hanoi Medical University Hospital were recruited into the study.

***Inclusion criteria*:**

- Subjects who were confirmed to be azoospermic by two semen analyses collected at least 2 weeks apart.

- Had sufficient relevant clinical history.

- Had serum hormonal profile measured by blood test and testicular size measured by ultrasound

- Diagnosed with NOA based on medical history, physical examination, semen analysis, hormonal profiles and testicular volume.

- Underwent microTESE procedure for sperm extraction and had a testicular specimen collected for histology evaluation.

***Exclusion criteria*:**

- Had normal spermatogenesis on histological evaluation.

- Azoospermic men after vasectomy procedure.

- Azoospermic men who underwent previous inguinal-scrotal surgeries.

- Azoospermic men with a history of testicular injuries.

**2. Methodology**

***Study design***

This is a cross-sectional observation study with convenience sampling. A total of 88 testicular specimens of 88 patients were collected for analysis.

***Study process***

Clinical history of all study patients were collected, including age, history of cryptorchidism, mumps orchitis, radiotherapy, chemotherapy and any previous surgical procedures. Azoospermia was confirmed by two semen analyses with an abstinence period of 3 to 5 days.

All patients had their serum hormonal profile and testicular size measured at Hanoi Medical University Hospital. Testicular volume was calculated using the formula: Volume = length * width * height * 0.71. Human karyotyping and AZF microdeletion analysis were also conducted at the Department of medical biology and Genetics – Hanoi Medical University. The karyotype was determined from leukocytes culture from peripheral whole blood that was scanned under electron microscope with Metafer scanning system. Microdeletion of AZF was determined by multiplex PCR technique using the AZPA_D kit.

All patients underwent microTESE procedure under general anethesia and during the procedure, a specimen was sent for histopathological evaluation at the Department of Histopathology, Hanoi Medical University.

Histopathology classification were based on five main histological patterns with the ascending severity of the permatogenesis disturbance[8].

- Normal spermatogenesis: Normal seminiferous tubule epithelium with full spermatogenesis in the entire biopsy

- Hypospermatogenesis (HYPOS): All stages of the spermatogenesis are present but there is a decline in the number of germ cells

- Germ cell maturation arrest (GCMA): Incomplete spermatogenesis, the arrest often occur at the spermatogonial stage and not beyond the spermatocyte stage.

- Sertoli cell only syndrome (SCOS): The seminiferous tubules contain no germ cells, only Sertoli cells are present.

- Seminiferous tubule hyalinization: The tubules contain either germ cells line or Sertoli cells and is usually accompanied by peritubular fibrosis

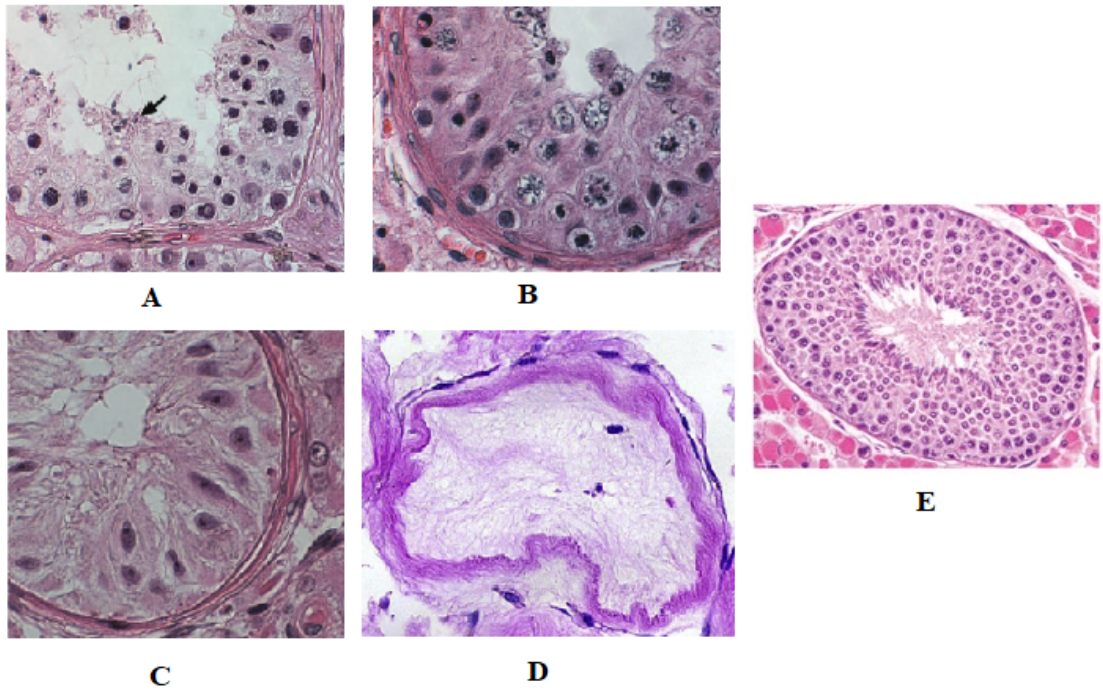



**Figure 1. Spermatogenic phenotypes:**
**(A): Hypospermatogenesis;**
**(B): Germ-cell maturation arrest;**
**(C): Sertoli cell only syndrome;**
**(D): Tubules hyalinization;**
**(E): Normal spermatogenesis**

### 3. Data processing

Data was processed using R software version 4.2.0 for Windows. Results were presented using quantity, percentage, mean and standard deviation. Variables were assessed for normal distribution using Kolmogorov-Smirnov test. Kruskal-Wallis test was used to determine the differences between the groups of histology pattern and etiology of NOA. Ordered logistic regression was used to determine the factors associated with testicular histopathology. All p-value of < 0.05 was considered to be statistically significant.

### 4. Ethical approval

This study was approved by the Ethical Review Committee of Hanoi Medical University (IRB764. Date of approval: 6th September 2022).

## III. RESULTS

**Table 1. Demographic characteristics of the subjects**

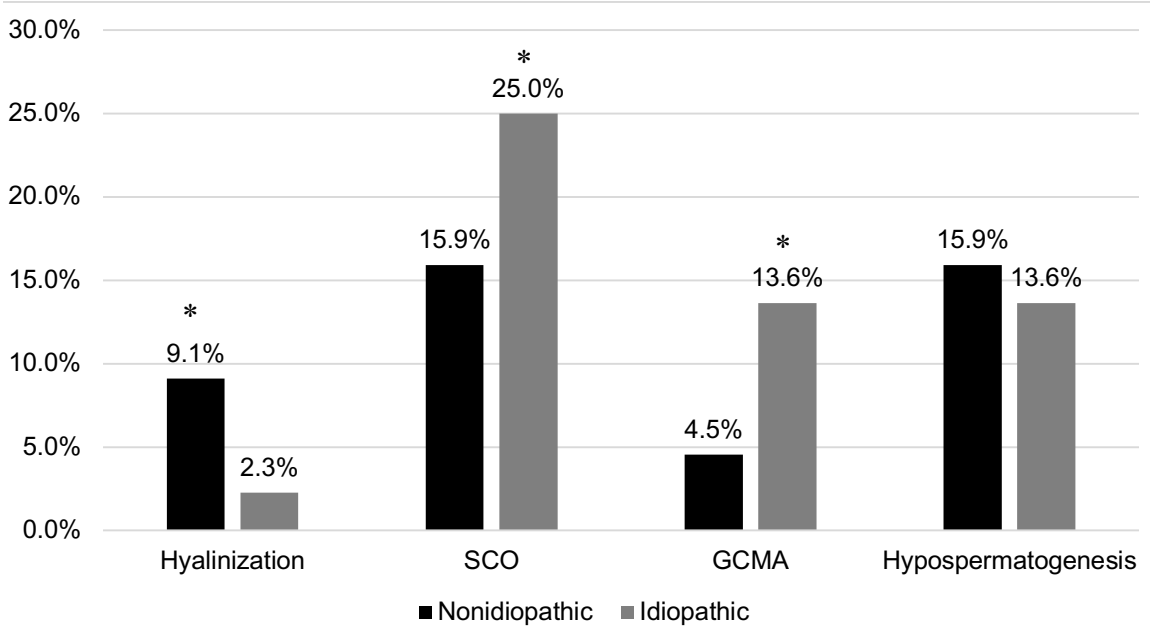
| Variables | N | % | Mean | SD |
|---|---|---|---|---|
| **Age (years)** | 88 | 100 | 30.36 | 5.22 |
| **Height (cm)** | 88 | 100 | 167.75 | 5.16 |
| **Weight (kg)** | 88 | 100 | 64.69 | 9.27 |
| **BMI (kg/m²)** | 88 | 100 | 22.95 | 2.9 |
| *<18.5* | 3 | 3.41 | | |
| *18.5-23* | 47 | 53.41 | | |
| *>23* | 38 | 43.18 | | |
| **History of mumps orchitis** | | | | |
| *Yes* | 21 | 23.86 | | |
| *No* | 67 | 76.14 | | |
| **Cryptorchidism** | | | | |
| *Yes* | 1 | 1.14 | | |
| *No* | 87 | 98.86 | | |
| **Type of infertility** | | | | |
| *Primary* | 81 | 92.05 | | |
| *Secondary* | 7 | 7.95 | | |

A total of 88 men were included in the study. The mean age was 30.36 years old. More than half (__%) had normal BMI. There were 21 patients (23.86%) with history of mumps orchitis and one (1%) with bilateral cryptorchidism. The majority of the patients (92.05%) had primary infertility (who never fathered a child).

**Table 2. Etiology of NOA**

| Etiology | n | % |
|---|---|---|
| Idiopathic | 48 | 54.55 |
| Non-idiopathic | 40 | 45.45 |
| Chromosome numerical abnomalies | 8 | 9.09 |
| Chromosome strutural abnormalies | 6 | 6.81 |
| AZF microdeletion | 9 | 10.22 |
| Mumps orchitis | 21 | 23.86 |
| Cryptorchidism | 1 | 1.14 |

A little more than half of the patients (n/N, 54.55%) had NOA of an unknown cause (idiopathic). Among these patients, mumps orchitis accounted for the largest proportion (n/N, 23.86%), followed by AZF microdeletion (n/N, 10.22%) and Klinefelter syndrome (n/N, 9.09%).




*: $p < 0.05$

**Figure 2. Testicular histopathology among idiopathic and nonidiopathic group**

The testicular histopathologies of idiopathic and nonidiopathic NOA was illustrated in Figure 1 with the SCOS being the predominant pattern among both groups. The proportion of SCOS and GCMA were higher in idiopathic NOA group compared to the nonidiopathic group ($p < 0.05$)

**Table 3. Comparison of demographic and clinical characteristics among testicular histopathology groups**

| Variables | Hyalinization | SCOS | GCMA | HYPOS | p* |
|---|---|---|---|---|---|
| | n = 10 | n = 36 | n = 16 | n = 26 | |
| Age (year) | 32.9 ± 5.12 | 28.69 ± 3.9 | 29.8 ± 3.01 | 32.11 ± 7.01 | 0.06 |
| BMI (kg/m$^2$) | 25.19 ± 4.43 | 22.69 ± 2.4 | 22.69 ± 2.49 | 22.61 ± 2.86 | 0.41 |
| Testicular volume (ml) | 5.73 ± 3.39 | 6.58 ± 3.44 | 11.77 ± 4.56 | 11.23 ± 3.52 | **<0.001** |
| Testosterone (nmol/l) | 9.33 ± 4.86 | 14.8 ± 6.84 | 16.57 ± 6.3 | 17.34 ± 9.03 | 0.054 |

| Variables | Hyalinization | SCOS | GCMA | HYPOS | p* |
|---|---|---|---|---|---|
| | n = 10 | n = 36 | n = 16 | n = 26 | |
| LH (mUI/l) | 13.81 ± 3.47 | 12.45 ± 6.37 | 5.41 ± 2.18 | 7.29 ± 6.16 | **<0.001** |
| FSH (mUI/l) | 30.9 ± 11.87 | 24.02 ± 11.97 | 9.34 ± 6.12 | 11.47 ± 10.56 | **<0.001** |

* p was calculated using Kruskal-Wallis test

Among the clinical characteristics, it is evident that LH, FSH and testicular volume were stratified according to the testicular histopathology. LH and FSH levels were significantly higher and the testicular volume was significantly lower along with the escalated severity of the spermatogenesis.

**Table 4. Ordered logistic regression for the association between predictors and histopathological pattern**

| Variables | OR | 95%CI | P |
|---|---|---|---|
| Age (Years) | 0.96 | 0.88 – 1.04 | 0.4 |
| FSH (mUI/l) | 1,071 | 1.01 – 1.12 | **0.011** |
| LH (mUI/l) | 1,003 | 0.09 – 1.11 | 0.95 |
| Testicular volume (ml) | 0.89 | 0.78 – 1.01 | 0.07 |

Ordered logistic regression model revealed that FSH was the only significant factor associated with the histological pattern of the testis. For each unit increase in the serum FSH concentration, the odds of having a more severe impaired stage of spermatogenesis increased by 7.1%. These results are further illustrated in Figure 3 with the probability of Hyalinization pattern increased with the elevation of FSH level, while SCOS pattern reached the highest probability with the level of FSH ranged between 20 and 40 mUI/l. Patients with normal FSH level had a high chance of hypospermatogenesis pattern.

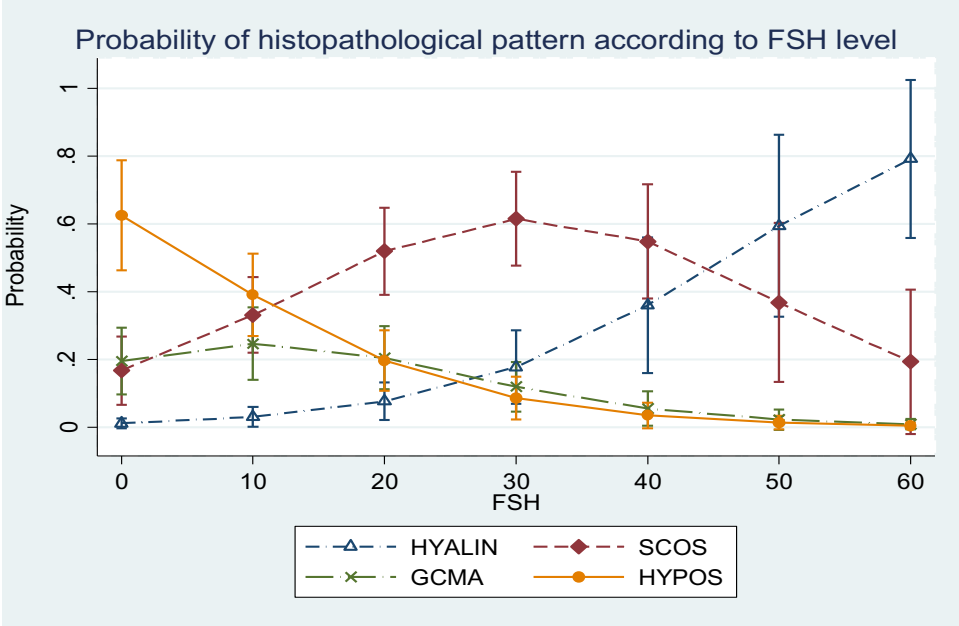



**Figure 3. Probability of histological pattern according to FSH level**

## IV. DISCUSSION

The discrepancy in the prevalence of male infertility is observed among populations around the world. This can be due to several etiological factors including social habits, genetics and environmental conditions.[9] In our study, we found that Klinefelter syndrome and AZF microdeletion accounted for 26.12% of all causes. Our results are silimar to previous studies on the genetic causes of NOA identified by routine analysis of a large population which reported the prevalence of about 13.7% for karyotype abnormalies and 8% for AZF microdeletions.[2,10] However, is it postulated that the prevalence of genetic mutations that contribute to NOA are underdiagnoses. Therefore, further studies using next-generation sequencing technologies are required to determine the genetic variants associated with idiopathic NOA. Beside genetic anomalies, our results showed high proportion of patients with history of mumps orchitis which caused testicular atrophy. Previous reports indicated that 30-87% of men were affected with mumps virus following bilateral orchitis.[11] Although mumps can be prevented by vaccination, in the past decade, there have been large outbreaks of mumps worldwide due to the decline in the effectiveness of the vaccine.[11] In addition, the majority of patients do not seek medical treatment after mumps orchitis, therefore, the prorpotion of men affected by testicular atrophy and subsequently decreased semen quality remains high. Unfortunately, currently there is no treatment for testicular atrophy after orchitis to recover the size or the spermatogenesis of the testis. Therefore, it is important to elevate the awareness of the population about mumps orchitis and encourage those with mumps orchitis to see a doctor as soon as possible.

For azoospermic infertile men, testicular biopsy is one of the important assessments. This procedure has both diagnostic and therapeutic significance.[8] Testicular histopathology provides pivotal information such as the stage

of spermatogenesis, the prognosis, or the presence of carcinoma in situ. In addition, spermatozoa can be harvested from the testis by testicular biopsy and testicular tissue can be cryopreserved for future ICSI. The procedure has allowed many patients with NOA to father their own biological children, especially men with genetic disorders.

In our study, the most prevalent histopathological pattern was SCOS (40.9%), followed by hypospematogenesis (__%) and GCMA (__%). These results are comparable with previous studies on testicular histopathology of NOA patients with the prevalence of SCOS, hypospermatogenesis and GCMA ranging from 50-76.3%, 13.6-32% and 14.2-29%, respectively.[5,12,13] In this study, our objective was to evaluate the impact of testicular histopathology on the outcome of intracytoplasmic sperm injection (ICSI In addtition, in this study, we observed that the proportions of SCOS and GCMA were higher in idiopathic NOA patients compared to nonidiopathic group. The opposite was observed among the hyalinization histology pattern. Das et al. also demonstrated the same difference on 224 NOA men.[7] Previously, it was postulated that the major causes of SCOS and GCMA genetic abnormalities such as Klinefelter syndrome or AZF microdeletion.[14,15] However, our results suggested that there are many unknown genetic variants that can disrupt complete spermatogenesis. Therefore, more powerful technologies should be utilized to identify the causes of NOA.

We also observed that the histopathological patterns differed significantly in the average testicular volume and serum LH and FSH concentrations. The average testicular volume was the lowest in those with severe disruption in the spermatogenesis. This result can be explained with the fact that the seminiferous tubules contribute to 70-80% of the testicular volume.[16] The damage or the absence of these tubules lead to the decrease of testicular size. FSH is a dimer glycoprotein produced in the pituitary. In men, FSH receptors are found in Sertoli cell of the testis and FSH facilitates the function of Sertoli cell. Sertoli cell in turn produced inhibin B, a peptide to control the secretion of FSH via a negative feedback mechanism. As a result, any damage to the seminiferous tubules or Sertoli cells will diminish the inhibin B concentration and, consequently, raise the FSH concentration.[17] Our results supported this mechanism. The FSH concentration was subnormal in hypospermatogenesis and GCMA groups (11.47 ± 10.56 mUI/l and 9.34 ± 6.12 mUI/l) but notably elevated in the hyalinization and SCOS groups (24.02 ± 11.97 mUI/l and 30.9 ± 11.87 mUI/l). This result is similar in previous reports of the association between testicular volume and hormonal level with testicular histopathology.[6,18,19]who already underwent a previous failure testicular fine needle aspiration.\nMethods: We evaluated a total of 82 azoospermic men, underwent testicular sperm extraction, referring to the Assisted Reproductive Technology Centre of the University of Florence, Italy between January 2008 and March 2017. A general and genital physical examination, scrotal and trans-rectal ultrasound, semen analysis, hormone measurements, including follicle-stimulating hormone, luteinizing hormone and total testosterone, were collected.\nResults: Successful sperm retrieval was obtained in 36 men of total (43.9%

In this study, the level of histopathological pattern was classified from mild to severe disruption of the spermatogenesis (from hypospermatogenesis to hyalinization). In the multinomial logistic regression model, only FSH concentration was significantly associated

with the change in the histological pattern. For each unit increase in FSH concentration, the odd of a more severe histopathological pattern increased by 7.1% (95%CI: 1.01-1.12). This association is further illustrated in Figure 3. For patients with FSH level of less than 20 mUI/l, the probability of hypospermatogenesis and GCMA pattern is much higher. While the chance of SCOS and hyalinization pattern were peaked at the FSH concentration of 20-40 mUI/l and above 40 mUI/l, respectively. Therefore, FSH concentration had a predictive value of the histopathological pattern in NOA patients.

## V. CONCLUSION

Sertoli-cell only syndrome and hypospermatogenesis were the most common histopathological patterns among NOA men. More severe histopathological damage associated with lower average testicular volume. Only FSH concentration was significantly associated with severity in terms of testicular histopathology. Each unit increase in FSH concentration (mUI/ml) was associated with a 7.1% increased odd of having more disrupted spermatogenesis.

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