DOI: 10.4081/aiua.2026.15833

ORIGINAL PAPER

# Association of testicular MRI findings with spermatogenic status and intratesticular sperm in patients with azoospermia or cryptozoospermia

**Soichiro Ogawa, Yusuke Kirihana, Kei Yaginuma, Satoru Meguro, Ryo Tanji, Kanako Matsuoka, Seiji Hoshi, Junya Hata, Yuichi Sato, Hidenori Akaihata, Motohide Uemura, Yoshiyuki Kojima**

Department of Urology, Fukushima Medical University School of Medicine, Fukushima, Japan.

**Summary** ***Background: The pathophysiology and presence of intratesticular sperm in patients with azoospermia are usually assessed based on clinical findings, hormonal evaluation, and ultrasonography; however, assessment based on these conventional examinations does not always yield an accurate diagnosis.***

***Objectives: We focused on magnetic resonance imaging (MRI) and aimed to investigate the association between testicular MRI findings, spermatogenic status, and the presence of intratesticular sperm in patients with azoospermia or cryptozoospermia.***

***Methods: Thirty-three patients (45 testes) who underwent preoperative testicular magnetic resonance imaging (MRI) and intraoperative testicular biopsy during varicocelectomy or testicular sperm extraction were included in this study. Associations between the apparent diffusion coefficient (ADC) values of testes and clinical parameters were analyzed.***

***Results: Among the 33 patients (45 testes) included in the analysis, normalized ADC values showed significant positive correlations with serum follicle-stimulating hormone (FSH) (rs = 0.366, p = 0.015) and LH (rs = 0.414, p = 0.006), and a significant negative correlation with Johnsen's score count (JSC) (rs = -0.377, p = 0.0088). No significant correlation was observed between ADC values and total testosterone (TT) concentration or testicular volume (TV). ADC values were significantly higher in testes without intratesticular sperm (p < 0.000005). Receiver-operating characteristic (ROC) analysis yielded an area under the curve (AUC) of 0.667 for predicting the presence of intratesticular sperm, using a cutoff ADC value of $0.382\times10^{-3}$ $mm^2/s$ (sensitivity 0.686, specificity 0.8).***

***Conclusions: These findings suggest that testicular MRI findings are associated with spermatogenic status and the presence of intratesticular sperm and may provide complementary information when combined with other clinical parameters.***

*KEY WORDS: Apparent Diffusion Coefficient (ADC); Intratesticular sperm; Male infertility; Magnetic Resonance Imaging (MRI).*

Submitted 15 June 2026; Accepted 17 July 2026

## INTRODUCTION

The prevalence of male infertility is gradually increasing worldwide (1). Infertility is a serious problem that not only prevents individuals from fulfilling their wishes to have and raise children, but also causes demographic shifts with broader social consequences (1, 2). Therefore, it is extremely important to improve diagnostic capabilities for patients with male infertility, thereby enabling appropriate treatment.

The general assessment of male fertility typically includes a medical interview to obtain information on medical history and regular medications. In addition, objective data from semen analysis, endocrinological tests and scrotal ultrasound findings are also useful. Among these assessments, *follicle-stimulating hormone* (FSH) levels and *testicular volume* (TV) are emphasized in routine practice to predict male fertility (3, 4). However, these factors are not always sufficient to evaluate spermatogenic status (5, 6).

Recent advances in imaging technology have improved the accuracy of qualitative assessment of organs. *Magnetic resonance imaging* (MRI) provides high-resolution images and has the potential to noninvasively assess the histological structure of the testes. In particular, *apparent diffusion coefficient* (ADC) values on MRI have been reported to significantly differ between obstructive and *non-obstructive azoospermia* (NOA) (7). However, the utility of MRI for assessing spermatogenic status in patients with conditions other than azoospermia has not been well studied. Therefore, the aim of this study was to investigate the association between testicular MRI findings, spermatogenic status and the presence of intratesticular sperm in patients with azoospermia or cryptozoospermia.

## MATERIALS AND METHODS

### *Study population*

Sixty patients aged ≥ 20 years with testicular varicocele underwent microsurgical subinguinal testicular varicocelectomy between February 2014 and January 2025. Seventy-one patients aged ≥ 20 years with azoospermia, cryptozoospermia, or *ejaculatory dysfunction* (EjD) underwent *testicular sperm extraction* (TESE) between February 2014 and January 2025. Every patient underwent a detailed medical interview, physical examination, and transabdominal and scrotal ultrasonography, in addition to laboratory testing with sex hormone profiling (FSH, *luteinizing hormone* [LH], *total testosterone* [TT], and *pro-*

*lactin* [PRL]). TV was calculated using an ellipsoid model of the testis, based on ultrasound measurements from the scrotal surface to obtain the width, length and height of the testis, and multiplying these dimensions by a factor of 0.71 (8). Patients underwent semen analysis in our outpatient clinic or prior to their visit. Azoospermia was diagnosed based on a minimum of two semen analyses. Patients with azoospermia or cryptozoospermia also underwent G-band analysis of somatic chromosomes. A final total of 33 patients (45 testes) were included in this study.

### *MRI examination*

All MRI images were acquired using a 1.5 Tesla scanner (*Optima MR450w or Signa HDxt, General Electric Healthcare, Chicago, United States*) with a pelvic phased array coil. Axial T2-weighted images were acquired using the following parameters: *time to repetition* (TR)/*time to echo* (TE), 2000-12000/82.2-111.1; section thickness, 4.5-8.5 mm; *field of view* (FOV), 200 × 200-360 × 360 mm; matrix size, 256 × 256-320 × 320. *Diffusion-weighted imaging* (DWI) sequences with b values of 0 and either 800 or 1000 s/mm$^2$ were obtained using the following parameters: TR/TE, 4462-12403/63.3-78; section thickness, 4.5-8.5 mm; FOV, 240 × 240-400 × 400 mm; matrix size, 80 × 90-256 × 256. ADC map was automatically created at the workstation and sent to the hospital's picture archiving and communication system.

### *Image analysis*

Elliptical *regions of interest* (ROIs) were defined to encompass each testicular parenchyma to the greatest extent possible, while avoiding extension beyond the testicular boundaries. The mean ADC value of each testis was calculated based on these ROIs. Similarly, elliptical ROIs were established within the bladder lumen, excluding the bladder wall, to serve as a reference. Bladder ADC values were used as an internal reference for normalization, as previously described by *Karakas et al.* (7) The normalized ADC value was calculated by dividing the mean testicular ADC value by the mean bladder ADC value.

### *Data collection*

The following data were collected and analyzed in this study: age, height, body weight; serum FSH, LH, TT, and PRL levels; left and right TV; initial diagnosis; histopathological results based on *Johnsen's score count* (JSC); and normalized testicular ADC values on MRI.

### *Testicular biopsy during micro-TESE*

In patients with NOA, uni- or bilateral micro-TESE was performed as reported previously (9). In patients with cryptozoospermia and repeated unsuccessful *intracytoplasmic sperm injection* (ICSI) using ejaculated sperm, and in patients with NOA, uni- or bilateral micro-TESE was performed to retrieve sperm from the testes for ICSI. A small fragment of testicular tissue was collected for histopathological evaluation.

### *Testicular biopsy during cTESE*

In patients diagnosed with *obstructive azoospermia* (OA), a 3-mm incision was made in the tunica albuginea of one testis, and a small fragment of testicular tissue was collected for sperm retrieval and histopathological evaluation.

### *Testicular biopsy during microsurgical subinguinal testicular varicocelectomy*

Subinguinal testicular varicocelectomy was performed as reported previously (10), through a 3-cm incision just caudal to the external inguinal ring under magnification with an operating microscope. The testis was delivered through the incision and a small fragment of testicular tissue was collected for histopathological evaluation.

Relationship between ADC values and clinical parameters

The correlation between normalized ADC value of each testis and FSH, LH, TT, JSC at testicular biopsy, and TV was evaluated.

### *Relationship between ADC values and presence or absence of intracytoplasmic sperm in each testis*

The ADC value of each testis and the presence of intratesticular sperm were examined. No intratesticular sperm was defined as the absence of sperm on both TESE and histopathological evaluation of the testicular biopsy. In patients who underwent microsurgical subinguinal testicular varicocelectomy, no intratesticular sperm was defined as the absence of sperm on histopathological evaluation of the testicular biopsy. *Receiver-operating characteristic* (ROC) analysis was then conducted to assess the ability of normalized ADC values to diagnose the presence of intratesticular sperm. *Area under the curve* (AUC) was calculated, and optimal sensitivity and specificity values were determined using the Youden index. ROC curves were generated and AUC, optimal sensitivity, and optimal specificity were calculated.

### *Statistical analysis*

Statistical analyses were performed in STATCEL® version 3 software (*add-in software for Microsoft Excel; OMS Publishing Inc., Saitama, Japan*). Spearman's rank correlation coefficient was calculated in Microsoft Excel 2021 (*Microsoft Corp., Redmond, WA*). ROC curve generation and calculation of AUC, optimal sensitivity, and optimal specificity were performed in EZR on R commander version 1.64 (*Saitama Medical Center, Jichi Medical University, Saitama, Japan*) (11). Values of $p < 0.05$ were considered statistically significant.

## Results

### *Patient selection*

Figure 1 shows a flow chart of patient selection. Patient characteristics are listed in Table 1. Based on the clinical evaluations, 12 patients were diagnosed with NOA, 3 with OA, 1 with EjD, 2 with cryptozoospermia, and 15 with testicular varicocele (left side, n = 14; right side, n = 1) (Table 2).

### *Correlations between normalized ADC values and clinical parameters*

In all patients (n = 33, 45 testes), the median (min-max) ADC value was 0.357 (0.285-0.6) ×10$^{-3}$ mm$^2$/s. Spearman's rank analysis demonstrated variable correla-

***Figure 1.***
*Flow chart of patient selection. Of the 131 patients who underwent microsurgical subinguinal testicular varicocelectomy or testicular sperm extraction (TESE), the final study cohort comprised 33 patients (45 testes) who underwent both preoperative testicular magnetic resonance imaging (MRI) and intraoperative testicular biopsy.*

*EjD: ejaculatory dysfunction; TESE: testicular sperm extraction; MRI: magnetic resonance imaging.*

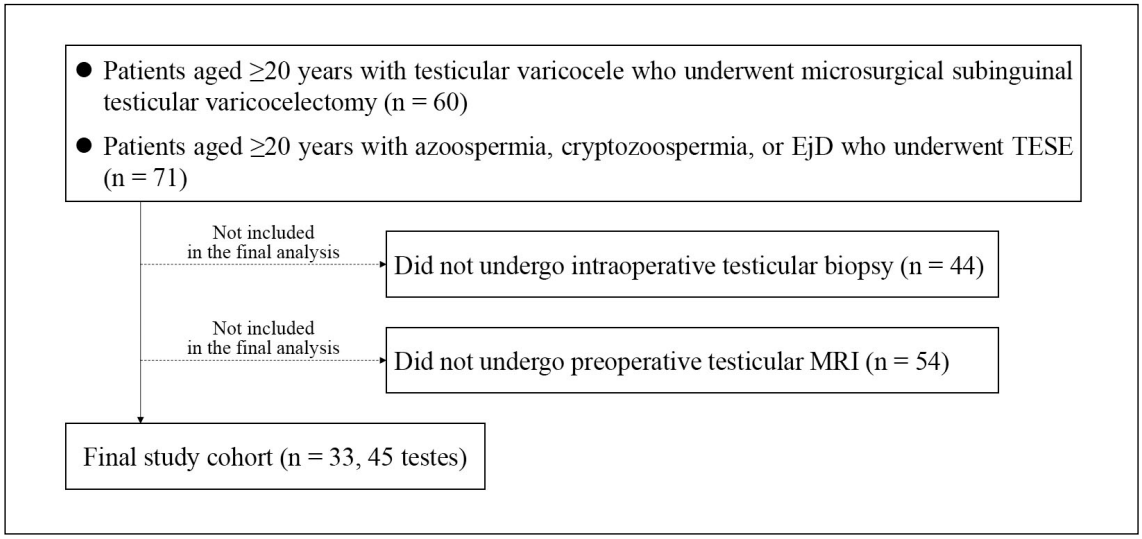


***Table 1.***
*Patient characteristics.*

| Variable | n | Median (minimum-maximum) |
|---|---|---|
| Age (y) | 33 | 33 (23-49) |
| BMI (Kg/m | 33 | 23.6 (18.1-29.1) |
| TV (mL) | 45 testes | 10.0 (0.66-25.1) |
| FSH (mIU/mL) | 33 | 7.5 (2.3-66.7) |
| LH (mIU/mL) | 33 | 4.9 (0.86-29.3) |
| PRL (mIU/mL) | 33 | 6.2 (2.6-12.9) |
| TT (ng/mL) | 33 | 4.9 (0.39-10.0) |

*BMI: body mass index; TV: testicular volume; FSH: follicle-stimulating hormone; LH: luteinizing hormone; PRL: prolactin; TT: total testosterone.*

***Table 2.***
*Surgical procedures.*

| Procedure | | n | |
|---|---|---|---|
| cTESE | unilateral | 3 | OA (n = 3) |
| micro-TESE | unilateral | 2 | EjD (n = 1), cryptozoospermia (n = 1) |
| | bilateral | 13 | NOA (n = 12), cryptozoospermia (n = 1) |
| Microsurgical subinguinal testicular varicocelectomy | unilateral | 15 | left side (n = 14), right side (n = 1) |

*cTESE: conventional testicular sperm extraction; micro-TESE: microdissection testicular sperm extraction; OA: obstructive azoospermia; EjD: ejaculatory dysfunction; NOA: non-obstructive azoospermia.*

tions between ADC values and clinical parameters. As shown in Figure 2a, a statistically significant positive correlation between ADC values and FSH was observed (rs = 0.366, p = 0.015). A statistically significant positive correlation between ADC values and LH was also observed (rs = 0.414, p = 0.006) (Figure 2b). No significant correlation between ADC values and TT was found (rs = 0.022, p = 0.886) (Figure 2c). A slightly negative but statistically non-significant correlation was observed between ADC values and TV (rs = -0.258, p = 0.089) (Figure 2d). In addition, as shown in Figure 2e, ADC values showed a statistically significant negative correlation with JSC (rs = -0.377, p = 0.009).

### *Predictive ability of ADC values for the presence of intratesticular sperm*

As mentioned above, FSH, LH, and JSC were correlated with normalized ADC values. These results suggest that normalized ADC values may reflect spermatogenic status. Therefore, we then examined whether ADC values could predict the presence of intratesticular sperm. The normalized ADC values of testes in which no sperm was observed (0.41 (0.289-0.6) $\times 10^{-3}$ $mm^2/s$) were significantly higher than those of testes in which sperm was observed (0.35 (0.096-0.5) $\times 10^{-3}$ $mm^2/s$) ($p < 0.000005$) (Figure 3). Representative ADC images of testes with and without intratesticular sperm are shown in Figure 4. No obvious visual differences were noted between the two groups, although quantitative analysis demonstrated significant differences in normalized ADC values. ROC analysis revealed an ADC cut-off value of 0.382 $\times 10^{-3}$ $mm^2/s$ (specificity, 0.8; sensitivity, 0.686), with area under the curve (AUC) of 0.667 (95% confidence interval [CI]: 0.428-0.907) for diagnosing the presence of intratesticular sperm (Figure 5). These results suggest that although the normalized ADC value was significantly correlated with FSH, LH, and JSC, it may be regarded as one of several informative parameters for assessing the likelihood of intratesticular sperm.

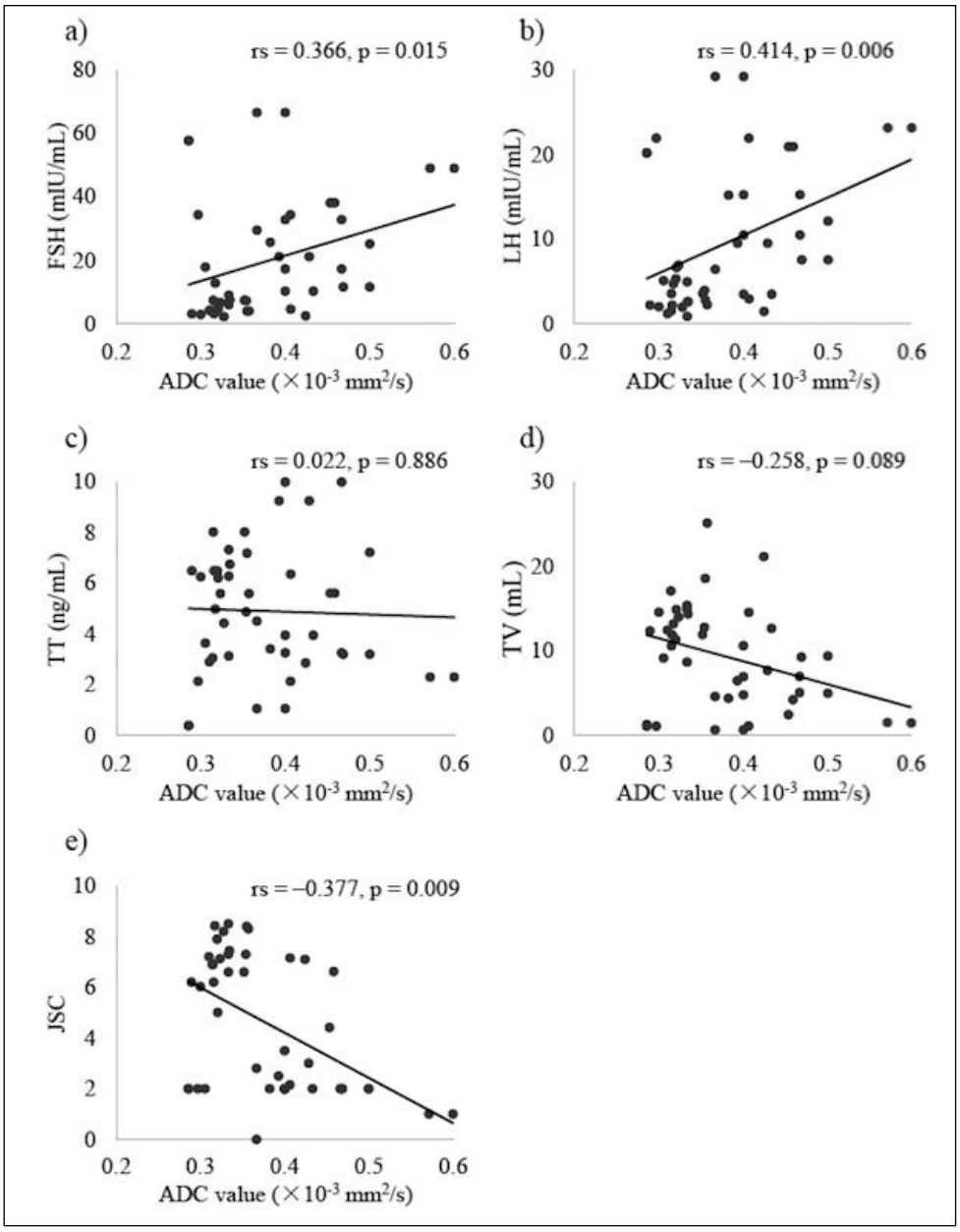


***Figure 2.***
*Correlations between ADC values and a) FSH, b) LH, c) TT, d) TV, and e) JSC.*

*ADC: apparent diffusion coefficient;*
*FSH: follicle-stimulating hormone;*
*LH: luteinizing hormone;*
*TT: total testosterone;*
*TV: testicular volume;*
*SC: Johnsen's score count.*

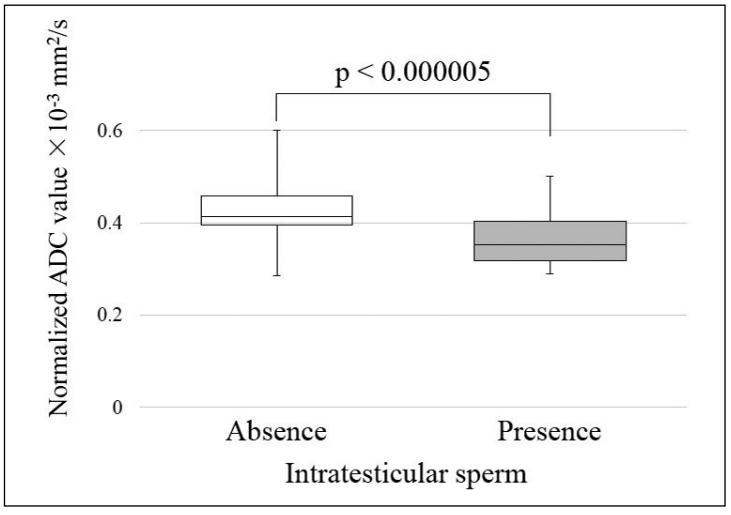


***Figure 3.***
*Box-and-whisker diagram showing normalized ADC values by presence/absence of intratesticular sperm.*

*ADC: apparent diffusion coefficient.*

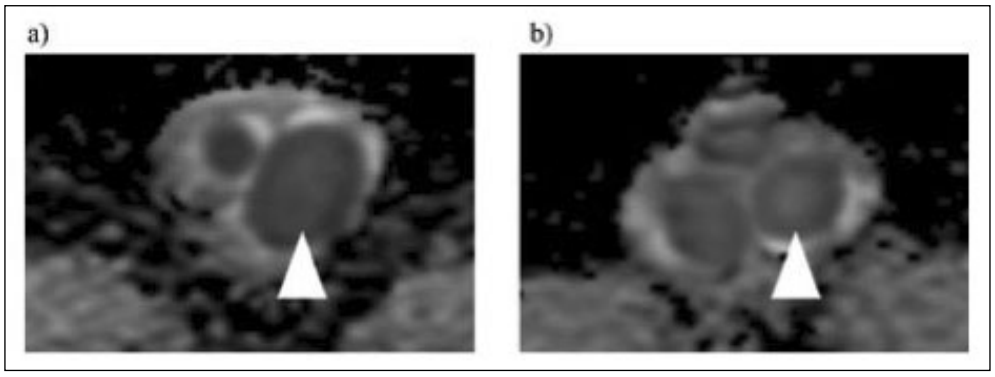


***Figure 4.***
*Representative ADC images of testes a) with and b) without intratesticular sperm.*
*White arrowheads indicate the left testis.*

*ADC: apparent diffusion coefficient.*

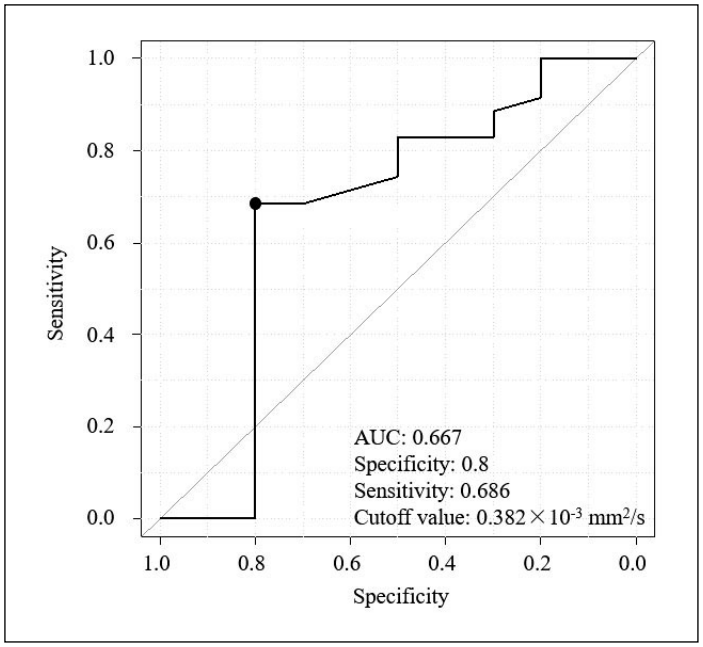


***Figure 5.***
*ROC curve showing performance of ADC values to predict the presence of intratesticular sperm.*

*ROC: receiver-operating characteristic;*
*AUC: area under the curve;*
*ADC: apparent diffusion coefficient.*

## Discussion

In this study, we evaluated whether testicular MRI could reflect spermatogenic status in patients diagnosed with male infertility. We found that FSH, LH, and JSC significantly correlated with normalized ADC values.

Diffusion-weighted MRI is an imaging technique that visualizes the motion of water molecules within tissue, and the ADC value is a quantitative measure of this motion (12, 13). Therefore, the ADC value is affected by cell density and intercellular matrix structure (13, 14). For example, a decrease in cell density leads to increased motion of water molecules in the intercellular spaces, resulting in a higher ADC value.

The significant positive correlation observed between ADC values and each of FSH and LH in this study suggests that primary testicular dysfunction with high gonadotropin levels is associated with decreased cell density. In testes with impaired spermatogenesis, the seminiferous tubules become narrower and their number decreases. *Regent et al.* demonstrated that in patients with NOA, MRI of the testes showed significantly higher ADC values compared with those with OA, which had histologically normal seminiferous tubules (15). Similarly, *Tsili et al.* compared the ADC values of testicular MRI between 48 patients with NOA and 18 age-matched control subjects (16), and found that ADC values were significantly higher in the NOA group than in the control group. That study did not analyze the direct relationship between ADC values and FSH levels; however, their results support those of the present study because FSH levels tend to increase with the severity of primary spermatogenic dysfunction.

In this study, a significant positive correlation was observed between LH levels and ADC values ($rs = 0.414$, $p = 0.006$). Elevated LH levels generally reflect impaired testicular function through hypothalamic-pituitary feedback regulation. Although alterations in Leydig cell function and intratesticular testosterone have been proposed as potential mechanisms underlying impaired spermatogenesis, these parameters were not evaluated in the present study. Therefore, the mechanisms responsible for the observed association between LH and ADC values remain to be clarified.

In this study, no significant correlation was observed

between TT and ADC values (rs = 0.022, p = 0.886). TT levels are susceptible to diurnal variations and metabolic influences within the body, as well as external factors such as health status, obesity, and stress.(17-19) Therefore, a single blood sample may not reliably reflect testicular function or cell density. In other words, because TT levels are influenced by systemic metabolism, they may not accurately represent the local state of the testes. This may explain the relatively weak correlation observed between TT and ADC values.

Interestingly, the ROC analysis using a cutoff value of $0.382\times10^{-3}$ $mm^2/s$ demonstrated moderate diagnostic performance (AUC = 0.667), suggesting that ADC values could provide supportive information in the assessment of spermatogenic status. However, this level of discrimination is insufficient for ADC values to be used as a standalone predictor of intratesticular sperm. Because ADC values reflect the averaged diffusion of water molecules within the ROI, encompassing seminiferous tubules, interstitium, blood vessels, and Leydig cells. Therefore, combining ADC values with hormonal profiles or other imaging parameters may improve overall assessment of spermatogenic status, although further prospective studies are required to confirm their clinical utility.

This study has several limitations that should be considered. First, the ADC value was calculated from a single ROI on one axial slice, which may not fully reflect focal areas of preserved spermatogenesis, particularly in patients with NOA. Future studies using multiple ROIs across different sections may improve diagnostic accuracy. Second, this was a retrospective study with a small and heterogeneous cohort. In particular, the number of patients with NOA was limited, precluding meaningful subgroup analyses, including comparisons according to sperm retrieval status. Therefore, larger prospective studies including more patients with NOA are necessary to verify whether ADC values can reliably diagnose the presence or absence of intratesticular sperm. Third, histological evaluation was based on biopsy specimens obtained from a limited area of the testis and therefore may not accurately reflect the spermatogenic status of the entire testis. Fourth, this study did not include a control group of healthy individuals.

Despite these limitations, our findings provide new insights into the association between diffusion-weighted MRI findings, spermatogenic status and the presence of intratesticular sperm and support further exploration of ADC values as part of a multimodal diagnostic approach.

## Declarations

***Ethical approval and consent for participate:*** *All study protocols were approved by the Ethics Committee of Fukushima Medical University (clinical approval No. 30291) and were conducted in accordance with the principles of the Declaration of Helsinki. The requirement for written informed consent was waived by the Ethics Committee, and information about the study was disclosed using an opt-out approach.*

***Availability of data and material:*** *The data that support the findings of this study are available from the corresponding author upon reasonable request.*

***Competing interests:*** *The authors declare no conflicts of interest.*

***Funding:*** *None.*

***Authors' contributions:*** *Soichiro Ogawa drafted the manuscript. Kei Yaginuma, Ryo Tanji and Satoru Meguro collected and analyzed the data. Yusuke Kirihana, Kanako Matsuoka, Seiji Hoshi and Yuichi Sato edited and revised the manuscript. Junya Hata, Hidenori Akaihata and Motohide Uemura managed data. Yoshiyuki Kojima supervised the whole study. All authors reviewed the manuscript.*

***Acknowledgments:*** *Not applicable.*

## References

*1. Huang B, Wang Z, Kong Y, et al. Global, regional and national burden of male infertility in 204 countries and territories between 1990 and 2019: an analysis of global burden of disease study. BMC Public Health. 2023; 23:2195.*

*2. Gong J, Wu Y, Qiu C, et al. . Global, regional, and national burden of diseases associated with male reproduction from 1990 to 2021: a systematic analysis with forecasts to 2050. BMC Public Health. 2025; 25:2404.*

*3. Schurich M, Aigner F, Frauscher F, Pallwein L. The role of ultrasound in assessment of male fertility. Eur J Obstet Gynecol Reprod Biol. 2009; 144 (Suppl 1):S192-8.*

*4. Kavoussi PK, Hudson K, Machen GL, et al. FSH levels and testicular volumes are associated with the severity of testicular histopathology in men with non-obstructive azoospermia. J Assist Reprod Genet. 2021; 38:3015-8.*

*5. Madani AH, Falahatkar S, Heidarzadeh A, et al. Sensitivity and specificity of serum FSH and testis size in predicting the existence of spermatogenesis in azoospermic infertile men. Andrologia. 2012; 44:205-9.*

*6. Andrade DL, Viana MC, Esteves SC. Differential Diagnosis of Azoospermia in Men with Infertility. J Clin Med. 2021; 10.*

*7. Han BH, Park SB, Seo JT, Chun YK. Usefulness of Testicular Volume, Apparent Diffusion Coefficient, and Normalized Apparent Diffusion Coefficient in the MRI Evaluation of Infertile Men With Azoospermia. AJR Am J Roentgenol. 2018; 210:543-8.*

*8. Sakamoto H, Saito K, Oohta M, et al. Testicular volume measurement: comparison of ultrasonography, orchidometry, and water displacement. Urology. 2007; 69:152-7.*

*9. Esteves SC, Miyaoka R, Agarwal A. Sperm retrieval techniques for assisted reproduction. Int Braz J Urol. 2011; 37:570-83.*

*10. Johnson D, Sandlow J. Treatment of varicoceles: techniques and outcomes. Fertil Steril. 2017; 108:378-84.*

*11. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013; 48:452-8.*

*12. Sinkus R, Van Beers BE, Vilgrain V, et al. Apparent diffusion coefficient from magnetic resonance imaging as a biomarker in oncology drug development. Eur J Cancer. 2012; 48:425-31.*

*13. Fornasa F. Diffusion-weighted Magnetic Resonance Imaging:*

*What Makes Water Run Fast or Slow? J Clin Imaging Sci. 2011; 1:27.*

*14. Hauge A, Wegner CS, Gaustad JV, et al. Diffusion-weighted MRI-derived ADC values reflect collagen I content in PDX models of uterine cervical cancer. Oncotarget. 2017; 8:105682-91.*

*15. Regent B, Skrobisz K, Kozak O, et al. MRI in the evaluation of the azoospermic male. Diagn Interv Radiol. 2020; 26:271-6.*

*16. Tsili AC, Sofikitis N, Astrakas L, et al. A magnetic resonance imaging study in etiology of nonobstructive azoospermia. Andrology. 2022; 10:241-53.*

*17. Ntorkou A, Tsili AC, Goussia A, et al. Testicular Apparent Diffusion Coefficient and Magnetization Transfer Ratio: Can These MRI Parameters Be Used to Predict Successful Sperm Retrieval in Nonobstructive Azoospermia? AJR Am J Roentgenol. 2019; 213:610-8.*

*18. Kutlikova HH, Durdiaková JB, Wagner B, et al. The effects of testosterone on the physiological response to social and somatic stressors. Psychoneuroendocrinology. 2020; 117:104693.*

*19. Kraemer WJ, Loebel CC, Volek JS, et al. The effect of heavy resistance exercise on the circadian rhythm of salivary testosterone in men. Eur J Appl Physiol. 2001; 84:13-8.*

**Correspondence**
*Soichiro Ogawa (Corresponding Author)*
soh@fmu.ac.jp
Department of Urology, Fukushima Medical University School of Medicine
1, Hikarigaoka, Fukushima 960-1295, Japan

*Yusuke Kirihana*
h-5111@fmu.ac.jp
*Kei Yaginuma*
uro-yagi@fmu.ac.jp
*Satoru Meguro*
s-meguro@fmu.ac.jp
*Ryo Tanji*
tanji422@fmu.ac.jp
*Kanako Matsuoka*
kanaco@fmu.ac.jp
*Seiji Hoshi*
uro-hosi@fmu.ac.jp
*Junya Hata*
akju826@fmu.ac.jp
*Yuichi Sato*
ysato@fmu.ac.jp
*Hidenori Akaihata*
hakai@fmu.ac.jp
*Motohide Uemura*
muemura@fmu.ac.jp
*Yoshiyuki Kojima*
ykojima@fmu.ac.jp
Department of Urology, Fukushima Medical University School of Medicine, Fukushima, Japan