Original Article

Preoperative Predictors for the Presence of Motile Spermatozoa in the Epididymis and Patency of Anastomosis during Microsurgical Vasoepididymal Anastomosis in Patients with Obstructive Azoospermia

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Abstract

Background: Following microsurgical vaso-epididymal anastomosis (VEA), anastomotic patency with sperm returning to the ejaculate is not always present and may even be delayed. The presence of motile spermatozoa is highly suggestive of future patency following surgery. **Aims:** We prospectively analyse the factors that could predict motile spermatozoa at the epididymis intraoperatively and predictors of patency in patients with obstructive azoospermia (OA) undergoing microsurgical VEA. **Settings and Design:** Department of Urology of a tertiary care centre in Northern India. It is a prospective observational study. **Materials and Methods:** Over a 2-year period (July 2019 to June 2021), 26 patients with idiopathic OA were enrolled in the study. Twenty patients underwent microsurgical VEA. Patients were divided into two groups based on the presence/absence of intraoperative motile spermatozoa. **Statistical Analysis Used:** Analysis of preoperative and intraoperative factors was done using the Mann–Whitney *U*-test, Chi-squared test and Fischer exact test. **Results:** Out of 20 patients, 5 (group 2) had intraoperative motile spermatozoa in the epididymal fluid and 15 (group 1) had nonmotile spermatozoa. Low luteinising hormone (LH) levels ($P = 0.01$) and high testosterone levels ($P = 0.05$) were the predictive of presence of motile spermatozoa in epididymal fluid. Mean follow-up was 9 months (6–18 months). Predictors of higher patency were grade 2 epididymis (firm, turgid and tense) ($P = 0.003$), low LH levels ($P = 0.03$), low sertoli cell index ($P = 0.006$), high sperm-Sertoli index ($P = 0.002$) and better surgeon satisfaction ($P = 0.01$). **Conclusion:** Low LH levels and high testosterone levels may be predictive of the presence of motile spermatozoa in epididymal fluid. Firm, turgid and tense epididymis, low Sertoli cell index, high sperm-Sertoli index and surgeon satisfaction suggest a greater chance of success after VEA for idiopathic azoospermia.

Keywords: *Male infertility, obstructive azoospermia, semen analysis, vasoepididymal anastomosis*

Introduction

One percent of the general population and 10%–15% of infertile males have azoospermia.[1] Approximately, 40% of azoospermic men with obstructive azoospermia (OA) are diagnosed with having epididymal obstruction.[2,3] In patients with OA, surgical reconstruction is an acceptable option for management. In the Indian scenario, the most prevalent causes of OA are genital tuberculosis, scrotal calcifications after infections, post-surgical and dense adhesions. A large number of cases

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are idiopathic in patients who have no history of previous vasectomy, inguinal or scrotal surgery/trauma or scrotal inflammation/infection.[4] Microsurgical vaso-epdidymal anastomosis (VEA) is the favoured reconstructive procedure for OA when the obstruction is found at the level of epididymis.[3,5] The procedure of microsurgical VEA has abandon rates of around 45% even in expert hands.[5] Compared to assisted reproduction techniques (ARTs) such as intracytoplasmic sperm injection (ICSI), surgical correction offers a long-term solution. It aims to correct the underlying pathology, improve the patient's self-image and allow the natural selection of the best sperm. It also avoids the need for repeated ART and sperm harvesting each time the individual wishes to have a pregnancy.[6] If we can find the factors which can predict the presence of motile spermatozoa in epididymal fluid, we may pave way for choosing our patients for this intricate procedure and also counsel them better. With this background, we designed this study to assess the preoperative factors (clinical, laboratory, ultrasound and cytological) which can predict the presence of motile spermatozoa in the epididymal fluid.

Materials and Methods

This was a prospective, single-centre study approved by the Ethics Committee (IEC No: INT/IEC/002735) of our tertiary care institute). All consecutive patients with OA, fulfilling all the inclusion criteria between July 2019 and June 2021 were included in our study. OA amenable for vaso epididymal anastomosis was performed in men having features of epididymal obstruction. Men with azoospermia having at least a palpable vas deferens and at least two semen analysis having normal fructose and volume more than 1.5 ml with centrifuged pellets showing no sperms and fine-needle aspiration showing maturation up to spermatozoa. The inclusion criteria included: (1) All sexually active adults males of age >21 years with a clinical diagnosis of OA undergoing VEA; (2) follicle-stimulating hormone (FSH) levels <7.6 mIU/ml and fine-needle aspiration cytology (FNAC) of testis showing maturation up to spermatozoa. This was based on the study by Schoor *et al*.[7] have shown that FSH is accurate predictor whether the azoospermia is due to obstruction or due to spermatogenic dysfunction. They found that FSH 96% of men with OA had FSH levels of 7.6 IU/L or less and 89% of men due to spermatogenic dysfunction had FSH of >7.6 IU/L; (3) Acceptance of the study by the patient after a valid informed consent. The study adhered to the principles of Helsinki Declaration with its amendments (2013). Convenient sampling was done owing to COVID-19 pandemic. Patients with OA due to vasectomy, congenital absence of vas deferens, non-obstructive causes of azoospermia (e.g. primary testicular failure), FNAC showing pathology other than maturation up to spermatozoa (e.g. maturation arrest, hypo spermatogenesis and Sertoli cell only) were excluded from the study. At the time of enrolment, detailed history was taken including sexual history, details of spouse, history of sexual disease and past history of surgery. Detailed physical examinations including general physical examination and clinical examination of the testis and epididymis were undertaken. The volume testis was noted using the Prader's orchidometer. The epididymal grading was done by a clinician experienced in performing VEA and graded as follows: Grade 1: Soft, flabby and collapsed, Grade 2: Firm, turgid and tense, Grade 3: Hard, turgid and tense. It was first validated in 5 cases (for inter and intra-observer reliability) by two surgeons (antiphospholipid syndrome to Aditya Prakash Sharma) performing vaso-epididymal anastomosis.

Radiological investigations included an ultrasound of the testis and epididymis for maximum epididymal diameter and testicular dimensions. The hormonal analysis includes measuring serum levels of testosterone, FSH and luteinising hormone (LH). Cytopathology included FNAC of bilateral testis for documentation of sperm quantity/density in a high-power field [Figure 1]. Two slides from each right and left testis were obtained, air-dried smears were stained with the May-Grünwald-Giemsa method, and 95% alcohol-fixed smears were stained with H and E stain wherever it is possible. 200–500 hundred consecutive spermatogenic and Sertoli cells were counted from a well spread area with good cellularity in a random manner using light microscope at ×40 high power field. Two slides from each right and left testis were obtained, air-dried smears were stained with the May-Grünwald-Giemsa method and 95% alcohol-fixed smears were stained with H and E stain wherever it is possible. Average number of each type of cell calculated from right and left testis cells. Three indexes calculated using these formulas:

Sertoli cell index = Sertoli cells/all spermatogenetic cells, including spermatozoa

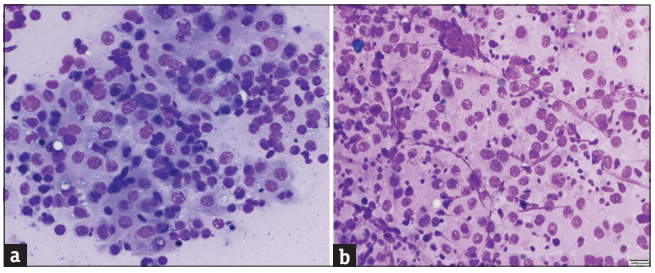

Figure 1: Fine-needle aspiration cytology slides showing (a) Abundant Sertoli cells with scanty spermatogenic cells. (b) Abundant spermatogenic cells with a few Sertoli cells

Spermatic cell index = Spermatozoa/all spermatogenetic cells including spermatozoa and excluding Sertoli cells

Sperm-Sertoli cell index = Spermatozoa/Sertoli cells

Surgical procedure

Scrotal exploration was performed under local anaesthesia. A longitudinal incision was given to expose the testis. The vas deferens was isolated and cut at the junction of straight and convoluted portion [Figure 2a]. Saline or methylene blue patency test was done. Vaso epididymal junction obstruction was diagnosed when there was a free flow of saline and patient having sensation of micturition or if catheterised there was the presence of bluish colouration on methylene blue testing. The epididymis was inspected for the appropriate site of anastomosis. The distal most site showing dilated tubules was preferred and the tunica was cauterised using minimal bipolar current. Incision over the tunica of epididymis was given using 11 number blade. The vas deferens was fixed to the tunica using two 8–0 prolene sutures. A suitable tubule was chosen and two 10–0 nylon sutures were preplaced into the tubules [Figure 2b]. The tubule was incised using ophthalmic keratome and the fluid emanating from the tubule was inspected under light microscope [Figure 2c]. Light microscopy findings were labelled as motile sperms, non-motile sperms or no sperms. The intussuscepting anastomosis was completed if the sperm was seen [Figure 2d]. If there was no fluid or the tubules were small, a multitubule anastomosis was performed. In case the epididymis is totally flaccid or cicatrised, the procedure was abandoned. The surgeon's subjective satisfaction with the anastomosis was graded from good to poor. All surgeries were unilateral.

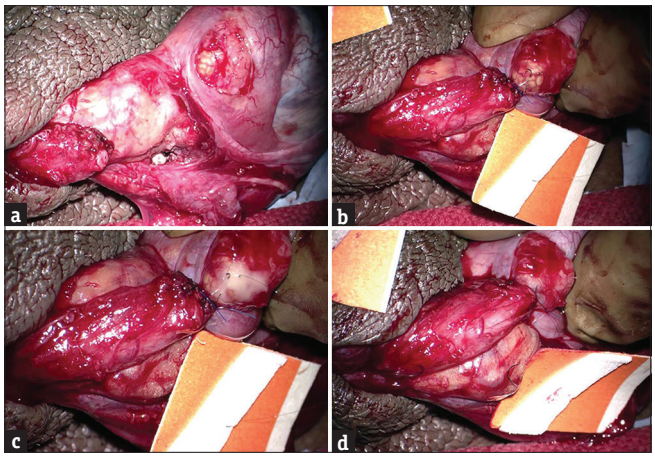

Figure 2: Surgical steps of Vaso-epididymal anastomosis. (a) Vas deferens cut at the junction of straight and curved portion and epididymal tubules exposed. (b) Vas secured to tunica of epididymis using 8-0 prolene sutures and 10-0 ethilon double armed sutures pre-placed. (c) Tubule punctured using ophthalmic keratome and seminal fluid seen. (d) Completed anastomosis

The patients were asked to abstain for 6 weeks and a semen analysis was performed at this point. Repeat analysis was performed 3 monthly till 1 year or till the appearance of the sperms whichever is earlier.

Statistical analysis was performed using the SPSS software version 26 for Windows (SPSS Inc., Chicago, IL, USA). The normality of data was assessed using Kolmogorov–Smirnov or Shaprio test. The descriptive statistics of continuous variables were expressed as median and interquartile range (IQR). For comparing a continuous variable between two independent groups at a particular time point, the Mann–Whitney *U*-test was employed. For comparing categorical variables at a particular time point, the Chi-squared test was employed if the expected value in all cells inside the contingency table was >5, else Fischer exact test was employed. Statistical significance was assumed when the probability value was <0.05.

Results

A total of 26 patients of OA who met the inclusion criteria provided consent for the study. Out of these 26, 6 procedures were abandoned due to various reasons (3 with epididymal scarring and three patients had complete vasal occlusion) and 20 underwent microsurgical VEA. The median age of patients in this study was 31.5 (IQR-30,33) years. Out of 20 patients, none had any comorbidity. Eight patients (40%) were chronic smokers with an average of 10 pack years, and three patients had a history of occasional alcohol intake. Two patients had a history of right inguinal herniorrhaphy.

All patients underwent unilateral microsurgical VEA under local anaesthesia, 13 patients on the right side and seven patients on the left side. Of the 20 patients where a VEA was performed, 5 had intraoperative motile spermatozoa in the epididymis and 15 had nonmotile spermatozoa. To compare factors associated with the presence or absence of motile spermatozoa on light microscopy in the epididymis, patients were divided into two groups, Group 1 had 15 patients (nonmotile spermatozoa) and Group 2 had five patients (motile spermatozoa).

The median age in Group 1 was 30 (IQR-30, 33) years and 32 (IQR-29, 36.50) in Group 2. The median duration of infertility in Group 1 was 3 (IQR-2, 5) years and 4 (IQR-2.25, 5) years in Group 2. Both mean age and mean duration of infertility were comparable in both groups [Table 1]. In FNAC, Differential cell counts and indices were comparable in both groups.

Both groups were comparable based on Ultrasound scrotum findings, i.e. testicular volume and epididymal

diameter between the two groups [Supplementary Table 1] Using an orchidometer, testicular volume was measured in both groups. Testicular volume was estimated to be slightly higher by ultrasound. In comparing surgeon satisfaction with anastomosis. All anastomoses made in group 2 were rated as good by the operating surgeon. In Group 1, 5 (33%) were rated as good, 6 (40%) as average, and 4 (26%) as poor on satisfaction rating.

On follow up 13 patients (65%) had patent anastomosis, 80% in group 2 and 60% in group 1. The semen analysis is given in the table below [Table 2].

For determining the predictors of patency of VEA, we compared patients who had patent anastomosis (Group A) and who did not have patent anastomosis (Group B). Epididymal grade 2, low LH levels, low Sertoli cells, low Sertoli cell index, and high sperm-Sertoli index were found in group A and were statstically significant [Tables 3 and 4].

Both groups were comparable based on ultrasound scrotum findings, i.e. testicular volume and epididymal diameters.

The median epididymal diameter was 1.00 (0.88, 1) mm in the patent group and 1.00 (0.33, 1) mm in the nonpatent group with a $P = 0.31$. Surgeon satisfaction was good in 10 (71%) on average in three (21.4%) patients in group A. In group B, 3 (33%) were average, and 4 (66%) were with poor satisfaction ($P = 0.01$) [Table 5].

Table 1: History, examination and laboratory parameters

	Group 1 (*n*=15)	Group 2 (*n*=5)	*P*
History and examination			
Age (years)	30 (30-33)	32 (29-36.50)	0.30
Duration of infertility (years)	3 (2-5)	4 (2.25-5)	0.73
Epididymal Grade 1, *n* (%)			
Right	6/15 (54)	0/5 (0)	0.26
Epididymal Grade 2, *n* (%)			
Right	2/15 (1)	5/5 (100)	
Left	7/15 (33)		
Testicular volume (mL), *n* (%)			
Right	15 (12-15)	15 (15-17.50)	0.44
Left	15 (12-20)	15 (12-17.50)	0.86
Hormonal workup			
FSH value (IU/L)	4.75 (3.31-5.50)	4.60 (2.61-6.00)	0.93
LH value (IU/L)	6.20 (4.95-6.42)	4.29 (2.95-5.10)	0.01
Testosterone value (mmol/L)	18.10 (13.80-20.40)	24.00 (18.16-25.80)	0.08

The values are median (IQR). FSH=Follicle-stimulating hormone, LH=Luteinising hormone, IQR=Interquartile range

Table 2: Post-operative semen analysis

Indices	Group 1	Group 2	*P*
ph	7.80 (7.55-8.00)	7.65 (7.52-8.00)	0.67
Volume (mL)	1.5 (1-2)	2 (1.75-2.50)	0.09
Sperm concentration (million/mL)	0.30 (0.00-0.56)	1 (0.25-2.45)	0.09
Total sperm count (millions/ejaculate)	0.50 (0.00-1.50)	2.00 (0.37-5.50)	0.14
Total motile (%)	10 (0-20)	10 (4-50)	0.55
Progressive motile (%)	0 (0-5)	5 (1-20)	0.14
Normal morphology (%)	0 (0-14)	46 (5-67)	0.06

Median (IQR). IQR=Interquartile range

Table 3: Demographic and laboratory parameters: Comparing patency

	Patent (*n*=13)	Not patent (*n*=7)	*P*
Demographics			
Age (years) (%)	32 (27-33)	30 (30-34)	0.64
Duration of infertility (years)	3 (2-5)	3 (2-8)	0.94
Epididymal Grade 1, *n* (%)			
Left	1 (7.6)	4 (57.1)	0.003
Right		1 (14.2)	
Grade 2, n (%)			
Right	12 (92.3)		
Left		2 (28.5)	
Testicular volume (mL)			
Right	15 (13.50-20)	12 (10-15)	0.08
Left	15 (12-20)	15 (10-15)	0.48
Hormonal workup			
FSH value (IU/L)	4.60 (1.85-5.35)	5.50 (3.90-6.17)	0.30
LH value (IU/L)	5.00 (4.04-6.21)	6.40 (4.95-7.02)	0.03
Testosterone value (mmol/L)	18.50 (15.92-20.60)	20.40 (13.80-24.50)	0.50

The values are median (IQR). FSH=Follicle-stimulating hormone, LH=Luteinising hormone, IQR=Interquartile range

Table 4: Fine-needle aspiration cytology findings

	Patent (*n*=13)	Not patent (*n*=7)	*P*
Differntial cell counts			
Sertoli cells (%)	34.48 (28.38-40.87)	51.30 (43.83-77.46)	0.001
Primary spermatocytes (%)	16.46 (11.20-18.32)	16.68 (4.44-18.19)	0.93
Spermatids (%)	5.80 (4.79-7.65)	7.33 (6.15-8.48)	0.21
Spermatozoa (%)	24.21 (22.70-35.66)	43.86 (34.87-49.70)	0.056
Indices			
Sertoli cell index	0.61 (0.41-0.81)	1.22 (0.77-3.59)	0.006
Spermatic cell index	0.67 (0.60-0.69)	0.54 (0.46-0.72)	0.35
Sperm-sertoli cell index	1.21 (0.86-1.83)	0.41 (0.14-0.71)	0.002

Table 5: Intraoperative parameters

	Patent (*n*=13)	Not patent (*n*=7)	*P*
Surgeon satisfaction			
Good	10/13	0/7	0.01
Average	3/13	3/7	
Poor	0/13	4/7	
Epididymal tubule diameter (mm), median (IQR)	1.00 (0.88-1)	1.00 (0.33-1)	0.31

IQR=Interquartile range

Discussion

Micro surgical reconstruction is a viable option for patients with epididymal obstruction. It corrects the underlying pathology and offers long term solution in terms of natural selection of best sperms, chance of natural conception and improves patient's confidence. It also obviates the need for repeated ART or at least sperm harvesting each time the individual wishes to contribute to a pregnancy.[6]

In our study, a total of 20 patients underwent microsurgical VEA. Both groups were comparable based on mean age, mean duration of infertility, physical examination, testicular volume, serum FSH and scrotal ultrasound findings.

In our study, we devised a novel Epididymal grading system which was defined preoperatively during the physical examination of patients. As Peng *et al*.[8] had shown that clinically distended epididymis is one of the predictors of success of VEA, if sperm production is normal and there is no intratesticular obstruction, the epididymis is expected to distend. Similarly morphology of tubule is important in determining VEA outcomes.[9] Accordingly, on physical examination, the epididymis was graded as 1, 2, or 3. All patients in the second group had grade 2 epididymis, i.e. firm, turgid and tense, and on the other hand, in group 1, seven patients had grade 2 epididymis. Although the comparison was not statistically significant, still a grade 2 epididymis was found to be predictive of patent anastomosis. 92.3% in the patent anastomosis group had grade 2 epididymis which is defined as a firm, turgid and tense epididymis favoring a successful anastomosis.

Men who have impared spermatogenesis are likely to have lower fertility rate even though they may have adequate patency of anastomosis.[10] They are likely to use ART more often then the patients who have normal spermatogenesis.[10] Pre-operative LH is also a potential predictor of pregnacny after VEA[11] with elevated LH pointing towards preimary damage to leydig cells.[12] Low testosterone or high LH levels were found in about 30% of men undergoing evaluation for infertility.[13] Testosterone exerts negative feedback on the hypothalamus and pituitary gland resulting in decreased production of Gonadotropin-releasing hormone, FSH and LH consequently maintaining testosterone in its optimal range.[12] In our study, low LH levels and higher testosterone levels in the group with intraoperative epididymal motile sperms were found. An optimal concentration of testosterone is required for spermatogenesis and it also has a role in capacitation. Thus, high normal testosterone could be a potential marker of the presence of motile spermatozoa in the epididymal fluid. Although this difference in testosterone was not found in patients with patent and nonpatent anastomosis. The LH levels were again significantly lower in the patent anastomosis group. Thus, this hormonal finding will need to be explored further in a larger sample cohort.

According to Srivastava *et al*.,[14] patients with normal spermatogenesis as compared to patients with hypospermatogensis or high Sertoli cell in FNAC had the presence of intraoperative spermatozoa at the epididymal end. In our study, we have included differential cell counts and indices in FNAC findings to predict motile spermatozoa at the epididymal end and patency. We have not found any significant difference between these findings in predicting motile spermatozoa at the epididymal end, but the low number of Sertoli cells, low Sertoli index, and high sperm-Sertoli index were significantly different in patent and nonpatent groups. These findings suggest the presence of a high number of spermatozoa as compared to a number of Sertoli cells. According to Han *et al*.[15] in testicular FNA, the combination of differential cell counts and indices may be useful for quantitating spermatogenesis, which can be used as a predictor of fertility in male fertility treatment when analyzed along with clinical assessment, levels of FSH, LH and testosterone. In our study, low LH levels and low Sertoli cell index were found to be associated with high patency rates.

The surgical expertise of the microsurgeon is important in determinig the outcome of VEA. Nagler and Rotman in their survey found that surgeons performing microsurgical vasovsostomy without dry training had a patency rate of 53% while those with laboratory practice had a patency rate of 89%.[16] In our study, the level of the surgeon's technical satisfaction with the culmination of the procedure was also significantly different between the two groups (P = 0.032).5 out of five patients with intraoperative motile spermatozoa had good surgeon technical satisfaction, and 4 out of these 5 had postoperative spermatozoa in semen analysis.

Table 6: Studies reporting the patency and its predictors after vasoepididymal anastomosis

	Number of patients	VEA done, number (%)	Patency, number (%)	Predictors of patency
Gautam *et al*.[3] (2015)	29	29 (100)	12 (50)	Motile sperm in the epididymal fluid, High surgeons' technical satisfaction
Kumar R *et al*.[4] (2019)	34	19 (55.8)	6 (31.5)	Motile sperm in the epididymal fluid
Hong K *et al*.[17] (2016)	81	62 (76.54)	41 (61)	Anastomosis sites, motile sperm in the epididymal fluid
Harza M *et al*.[18] (2014)	65	36 (55.38)	28 (77.7)	Low FSH levels, motile sperm in the epididymal fluid
Kumar R *et al*.[19] (2010)	24	23 (95.80)	11 (47.82)	Motile sperm in the epididymal fluid, bilateral surgery
Our study	26	20 (76.92)	13 (65)	Epididymal grading, low LH value, low Sertoli cell index, high Sperm-Sertoli index and good surgeon's technical satisfaction

VEA=Vaso-epididymal anastomosis, FSH=Follicle-stimulating hormone, LH=Luteinising hormone

Peng *et al*.[8] reported that using longitudinal suture placement intussusception VEA, overall patency rate of 71.7%, with 80.5% for patients who underwent bilateral surgery and 41.7% for unilateral cases. In our study, the overall patency rate was 65% at a median follow-up of 6 months, which is lower than in other studies. This could be due to the performance of unilateral VEA in our study.

Regarding patency, after VEA, we defined the mere presence of sperm in postoperative semen analysis as a patent. In various studies, the patency rates have been defined differently. In a systematic review on the patency rates, Yoon *et al*. found that 10 studies defined patency as the presence of spermatozoa similar to our study, 4 defined it as the presence of motile spermatozoa. Other studies defined patency in terms of the following threshold spermatozoa concentrations: $>10^4$ spermatozoa per ml (5 studies); $>10^5$ spermatozoa per ml (3 studies); $>10^6$ spermatozoa per ml (1 study); $>10^7$ spermatozoa per ml (3 studies) and $> 10^8$ spermatozoa per ml (1 study).[16] The association of patency was found with low LH levels, good surgeon satisfaction, and low Sertoli cell index. Table 6 shows the comparison of studies reporting patency rates and its predictors.[3,5,17-19]

Overall, four factors were found to have an association with intraoperative motile spermatozoa and patency, i.e. epididymal grading, low LH value, low Sertoli cell index, and good surgeon's technical satisfaction during surgery. Limitation of the study was small sample size. Also, the generalisability of the study is limited by the fact that ICSI is a viable alternative in such couples who can afford the same.

Conclusions

Microsurgical VEA remains a challenging and surgically demanding surgery. In patients with OA who are undergoing microsurgical VEA, low LH levels and high testosterone levels may be predictive of the presence of motile spermatozoa in the epididymal fluid.

We have developed a novel epididymal grading system to help urologists or andrologists in determining the probability of patency in patients with epididymal obstruction. Firm, turgid and tense epididymis, low LH levels, low Sertoli cell index, high sperm-Sertoli index and better surgeon satisfaction may suggest a greater chance of success after a vaso-epididymal anastomosis for idiopathic azoospermia.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Data availability statement

Data will be made available from the corrresponding author at a reasonable request.

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Supplementary Table 1: Ultrasound findings			
	Group 1	**Group 2**	***P***
Median testicular volume (IQR) (mL)			
Right	14.44 (11.85-16.45)	16.45 (13.86-17.71)	0.19
Left	15.54 (12.63-18.46)	16.20 (12.24-19.18)	0.86
Median epididymis head diameter (IQR) (mm)			
Right	10 (8-10)	10 (8.5-10)	0.93
Left	10 (10-11.50)	10 (9.40-12)	1.00
Median epididymis body diameter (IQR) (mm)			
Right	4 (3.90-5.80)	4.8 (4.00-5.75)	0.39
Left	4.5 (3.20-5.50)	5.3 (4.50-6.00)	0.23
Median epididymis tail diameter (IQR) (mm)			
Right	4 (4-6)	3.8 (2.90-6.00)	0.35
Left	4 (4-4)	4.5 (3.75-6.00)	0.35

IQR=Interquartile range