

RELATION BETWEEN SEMINAL FLUID VOLUME AND GRADE OF VARICOCELE
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INTRODUCTION

1.1 The scrotum

is pouch of skin contains the testes, epididymis and spermatic cord. It has three layers from superficial to deep: skin, dartos muscle and Colles fascia (continuous antero-superior with the anterior wall membranous fascia-Scarpa's fascia). It is divided into two compartments by a midline fibrous septum (median raphe). Each compartment contains a testicle, epididymis and proximal spermatic cord.^[1]

1.2 Testis

Ovoid reproductive and endocrine organs, Each testis has an upper pole (usually tilted slightly anteriorly) and a lower pole, The left testis lies lower than the right in 85% of subjects, The right and left testes are separated by a fibrous median scrotal septum, which is deficient superiorly. Average dimensions of testes are 5 cm (length), 2.5 cm (breadth), 3 cm (antero-posterior) in adult persons, Each testicle in an adult weighs between 10 and 19 grams, The normal adult testicular volume is 15–20 mL, Or $(18.6 \pm 4.8 \text{ mL})$ calculated from the formula: $\text{length} \times \text{width} \times \text{depth} \times 0.52 = \text{volume}$. A total volume (both testis) of $> 30 \text{ mL}$ is indicative of normal function. A testis volume $< 12 \text{ mL}$ is associated with lower testicular function,^{[2]&[3]}

- The seminiferous tubules drain to the rete testis (have an appearance consistent with a space used to collect seminiferous tubular fluids and spermatozoa before being dispersed into the various separate openings of the developing efferent ductules).^[2]

Each testis is invested by three coats, which are from outside inwards.

- ❖ The tunica vaginalis, is a remnant of the fetal processus vaginalis. which has visceral and parietal layers,
- ❖ the tunica albuginea, a dense fibrous capsule that is thickened posteriorly to form a fibrous septum known as the mediastinum testis; fibrous septa stretch from the mediastinum into the testis, dividing it into 200–300 seminiferous tubules.
- ❖ A plexus of blood vessels and a loose connective tissue make up the vasculosa.

LAYERS OF THE SCROTUM:

“Some Dangerous Englishmen Called It The Testis”

S-Skin

D-Dartos muscle and fascia

E- External Spermatic fascia

C- Cremasteric fascia

I- Internal Spermatic fascia

T- Tunica vaginalis

T- Tunica albuginea

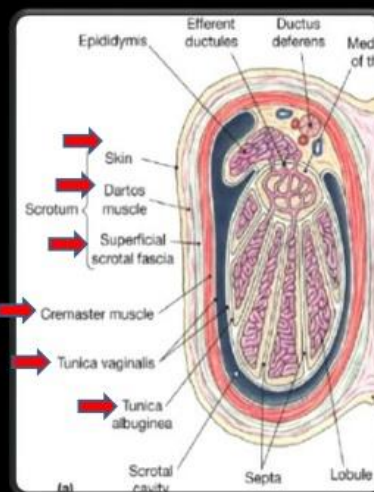


Figure 1: Anatomical layers of the scrotum.

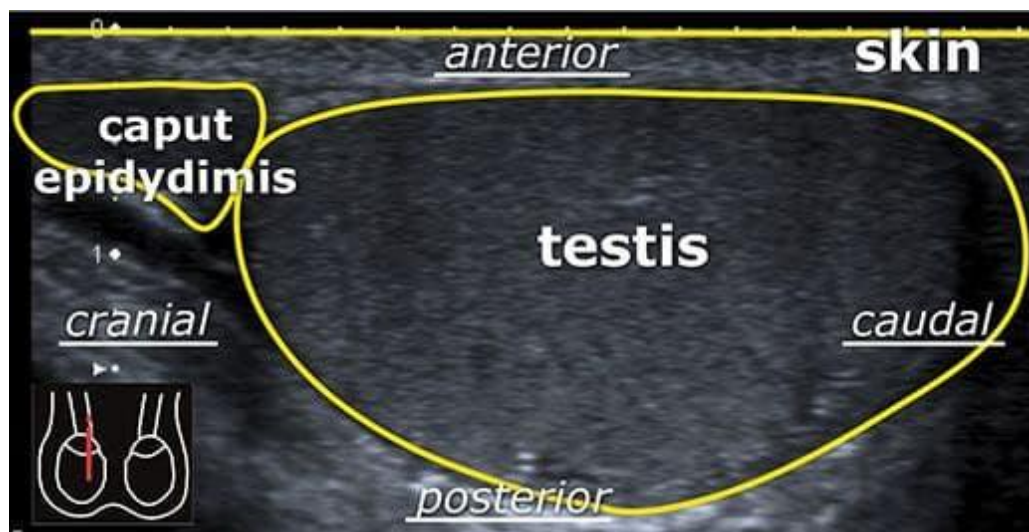


Figure 2: grey scale testicular image.

1.3 Blood supply

- At the level of the renal artery, the testicular artery emerges directly from the aorta.
- Branches of the inferior vesical artery and inferior epigastric artery supply the epididymis and spermatic cord.
- The external pudendal branch of the femoral artery supplies the scrotum.^{[4]&[5]}

course along the route of the testicular vessels to reach the para-aortic lymph nodes at the level of the L2 vertebra.^[6]

- while the scrotum drains to the lymph nodes in the inguinal area.^{[6]&[7]}

1.4 Lymphatic drainage

- Of the structures comprising the male external genitalia, the testes are the only ones that do not drain to the inguinal lymph nodes. Its lymphatics

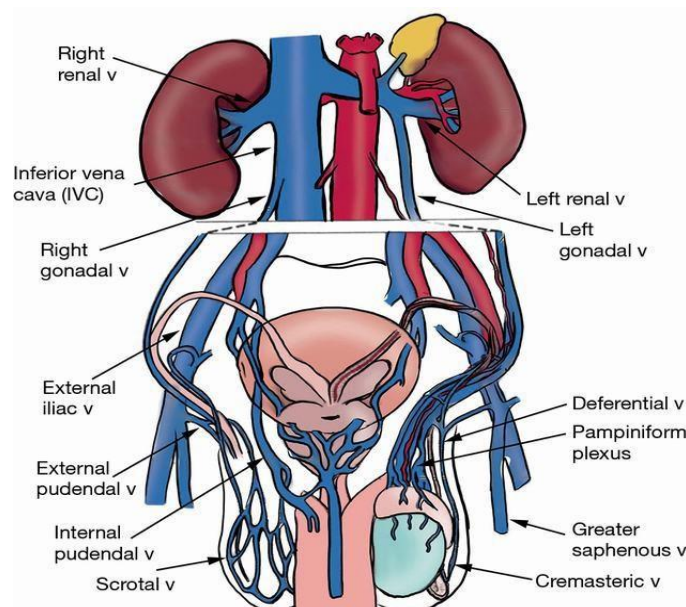


Figure 3: scrotal arterial and venous plexuses.

1.5 Venous drainage of the scrotum

The superficial network drains the scrotum, and these veins mainly follow the arteries, with the external pudendal branches draining into the great saphenous vein and the internal pudendal branches draining into the internal iliac vein.^[7]

The pampiniform plexus, a series of 10–12 small veins that drain the testis and epididymis, coalesce to form the gonadal vein, which empties into the renal vein on the left or the inferior vena cava (IVC) on the right. The pelvic plexus receives blood from the deferential vein., and the inferior epigastric vein drains into the cremasteric vein These routes aren't set in stone; there's a lot of variation amongst people when it comes to venous drainage.^[8]

1.6 Varicocele

The abnormal dilatation, compressibility, and tortuosity of the pampiniform plexus, resulting in retrograde flow into the internal spermatic vein.^[8]

1.6.1 Diagnosis

Physical examination and scrotal Doppler ultrasound are two ways to detect varicoceles being the most widely used techniques.

1.6.1.1 Clinical examination

is performed by inspection and palpation of the patient's scrotum in the standing position while in the relaxed position and while Valsalva is induced. Varicocele grading is based on the ability to visualize and/or palpate the varicocele in both the relaxed state and while inducing Valsalva. Grade I varicoceles are palpable only with Valsalva, grade II varicoceles are palpable without Valsalva, and grade III varicoceles are readily visible through the scrotal skin.^[9]

The diagnosis of varicocele is subject to significant inter-examiner variability, which is primarily determined by the level of competence of the examiner. As a result, with Doppler ultrasound diagnosis has become very common.^[10]

Clinical varicoceles is identified as varicoceles diagnosed by physical examination and confirmed by ultrasonography. Subclinical varicoceles were classified as those diagnosed solely by ultrasonography.

In the diagnosis consider the following.

- Left renal vein compression, caval compression, or tumor invasion in an elderly man.
- Valsalva is needed for the detection of small varicoceles.^[11]

1.6.1.2 Imaging

General Features

- Best diagnostic clue
 - Dilated serpiginous veins behind superior pole of testis on color Doppler US.
 - Distention secondary to retrograde flow with Valsalva.
- Location: Dilated veins in cremasteric plexus, vein of vas deferens, internal spermatic vein
- Size: Multiple veins in pampiniform plexus > 2-3 mm, ↑ size with Valsalva
- Morphology: Tortuous vascular channels (dilated veins).

Ultrasound Features

appear as multiple hypoechoic serpiginous tubular structures, initially superior and lateral, later posterior and inferior to testis containing low-level echoes if flow slow.

Color Doppler; Prominent color flow within vessels with Valsalva secondary to retrograde flow.^[12]

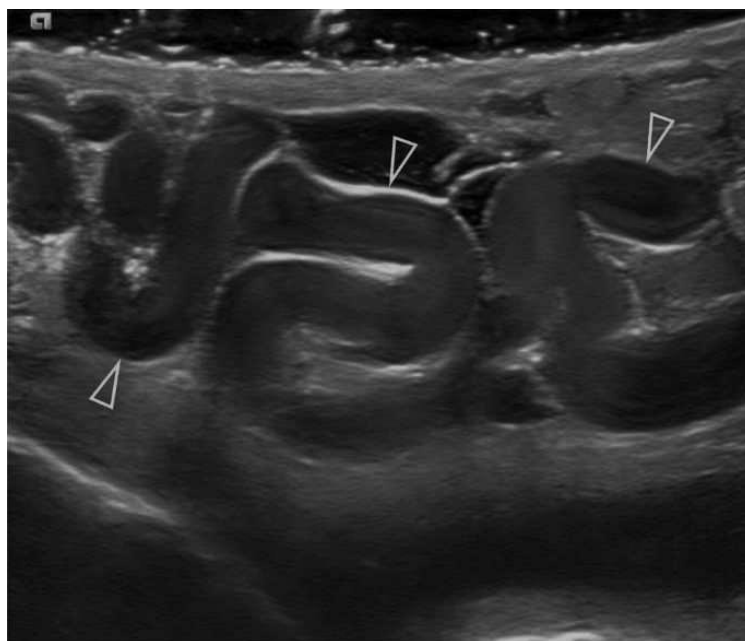


Figure 4: grey scale ultrasound image of varicocele.

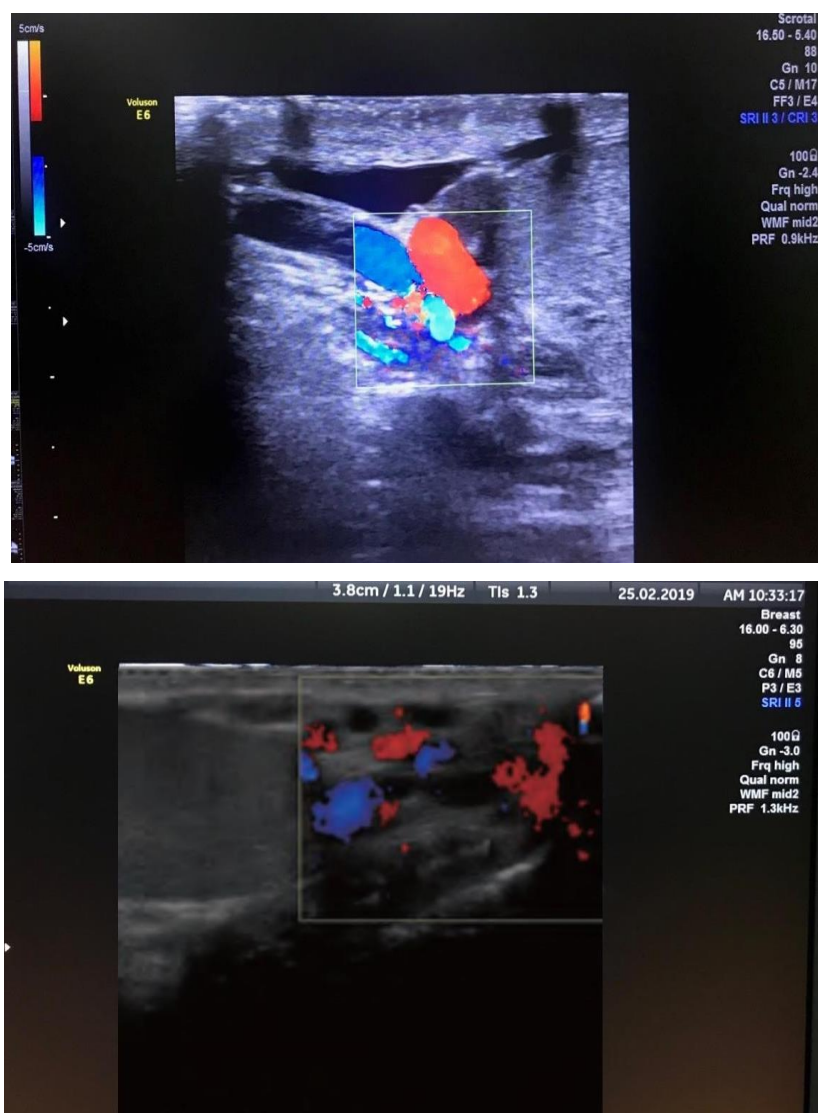


Figure 5: color Doppler ultrasound image of varicocele.

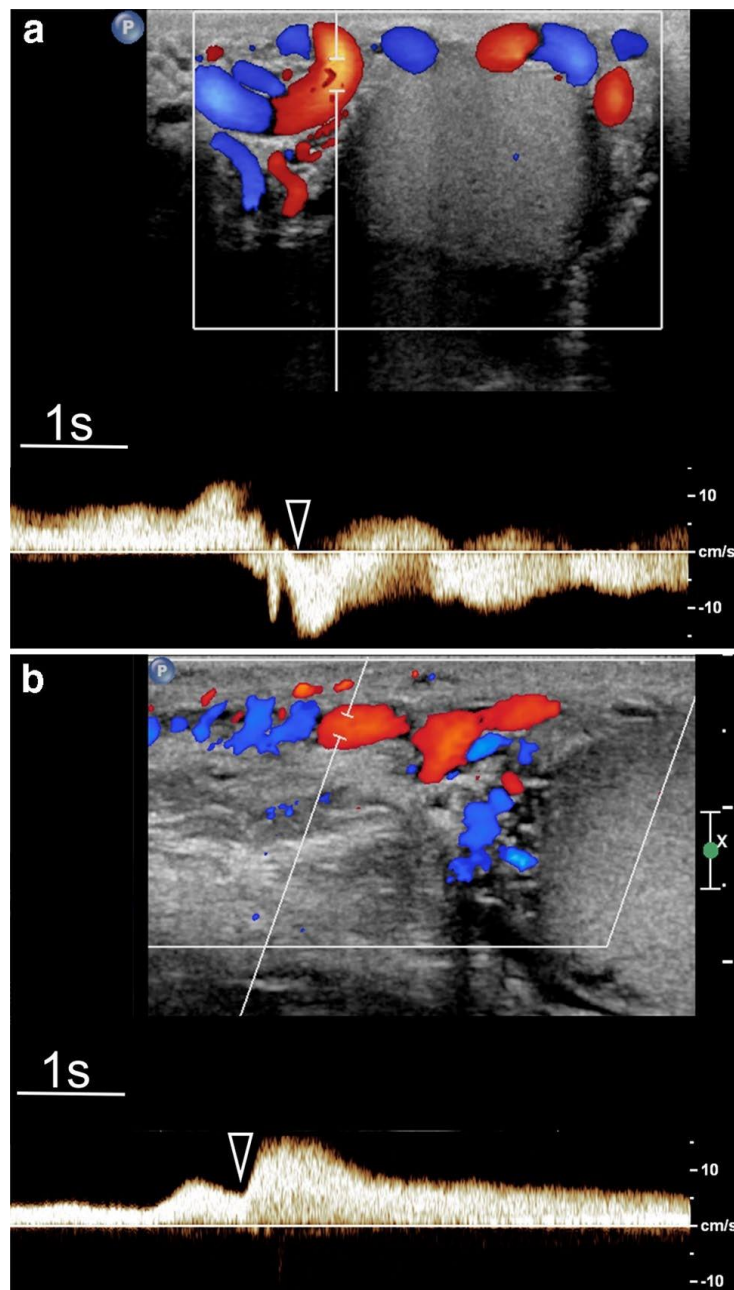


Figure 6: Spectral Doppler analysis in varicocele. Changes of reflux while standing during Valsalva (arrowhead). a Flow inversion. b Flow increase showing a plateau throughout Valsalva. In both cases reflux persists for more than 2 second.

1.6.2 Types of varicocele

1- Main or idiopathic: Caused by an inadequate or absent valve at the level of the left renal vein on the LT side and the inferior vena cava on the right side.^[13]

2. Secondary Varicocele

characterized by: tumor compression of the left renal vein, aberrant renal artery, obstructed renal vein, hydronephrosis, cirrhosis, so this should raise suspicion in cases of.

- 1-Older patient
- 2-New onset
- 3-Right side

4-No change in size with provocative maneuvers so we should check the left renal vein if there is non-compressible varicose vein.^[14]

1.6.3 Epidemiology

(a) grade II and III (also called clinical varicocele) : in 8–15% of adult males, in 21–39% of infertile men.

(b) grade I (also called subclinical varicocele) : in 40–75% of infertile male.

1.6.4 Mechanism by which affecting fertility

- I. Temperature rise in the area.
- II. Toxic compounds are excreted by the adrenal gland (countercurrent exchange of norepinephrine from

refluxing renal venous blood into testicular arterial blood at the level of the pampiniform plexus)

III. Changes in the function of the Leydig cell.

IV. Venous reflux causes venous hypertension and stasis, resulting in hypoxia of germinative tissue.

1.6.5 Clinical Presentation

- scrotal pain, scrotal swelling with “bag of worms” quality
- abnormal spermatogram: impaired motility, immature sperm, oligospermia.^[15]

1.6.6 Distribution

left side (78%), bilateral (16%), right side (6%)

left-sided prevalences due to.

The left testicular vein is longer than the right;

The left testicular vein joins the left renal vein at an angle (perpendicular)

The left renal vein in certain men may be compressed by the left testicular artery or a feces-filled descending colon.^[13]

The varicocele can be sub divided according to the reflux on color Doppler study with bidirectional in relaxing & straining or Supine & erect.

❖ Shunt type (86%): insufficient distal valves allow spontaneous & continuous reflux from the internal spermatic vein (retrograde flow) into the cremasteric vein & vein of the vas deferens (where flow is orthograde) via collaterals., this cause sperm quality diminished, clinically plexus type (grade II & III) of medium-sized & large varicoceles continuous reflux during Valsalva maneuver.

❖ Stop type / pressure type (14%): intact intrascrotal valves allow only a brief period of reflux from the spermatic vein into the pampiniform plexus under Valsalva maneuver, in this type sperm quality normal clinically central type (grade 0 & I) the subclinical & small varicocele cause short phase of initial retrograde flow.

Ultrasound is almost 100 percent sensitive and specific which identify the diameter of the dominant vein in upright position at the inguinal canal.^[16]

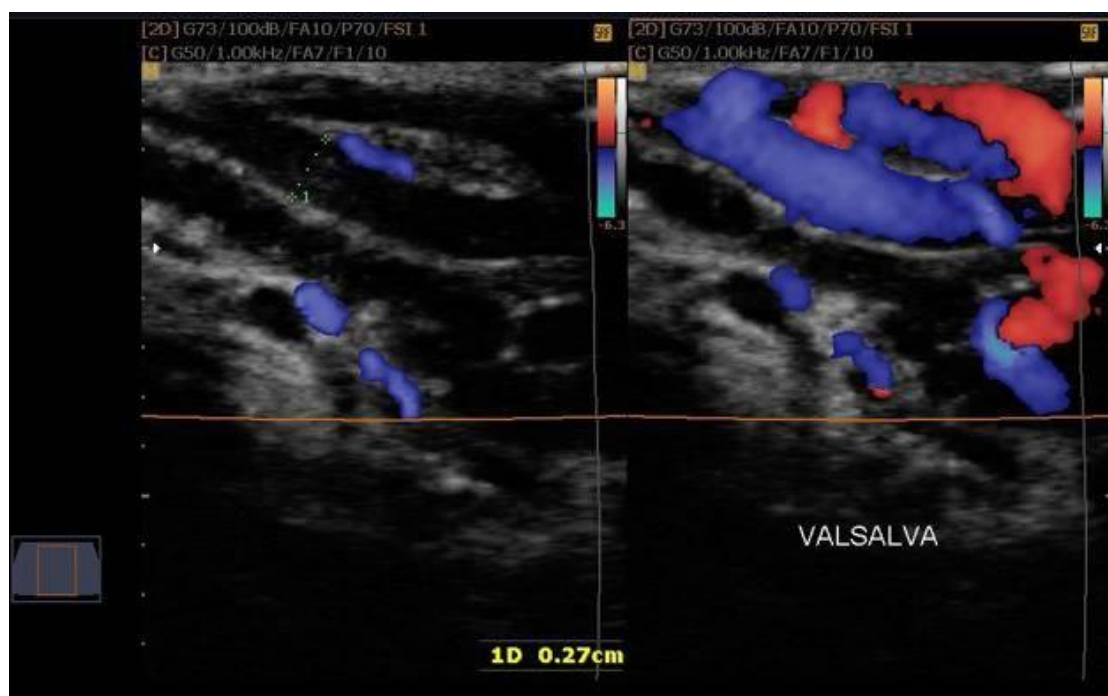


Figure 7: Example Of Measuring Varicocele.

1.6.7 Differential diagnosis

- Tubular Ectasia of Rete Testis: dilated seminiferous tubules within mediastinum testis, often associated with epididymal cyst, No flow on color Doppler (distinguish from intratesticular varicocele).
- Hematocele/Pyocele: complex fluid collection enveloping testis, conforms to testis, no color Doppler flow, clinical history aids diagnosis (trauma, fever, scrotal pain).
- Epididymitis: hypoechoic epididymis, flow on color Doppler.
- Hernia: echogenic fat (mesentery) &/or bowel displaces testis, may be accentuated with Valsalva,

no flow at color Doppler, check for gut signature, bowel peristalsis.^[17]

1.6.8 Treatment and prognosis

- Surgery.
- Interventional Radiology : Transcatheter spermatic vein occlusion

Treatment increases sperm production in up to 53% of cases.^{[18][19]}

1.7 Seminal Fluid

During ejaculation, semen is produced from a concentrated suspension of spermatozoa, stored in the

paired epididymides, mixed with and diluted by fluid secretions from the accessory sex organs. It is emitted in several boluses.^[20]

Precise measurement of volume is essential in any evaluation of semen, because it allows the total number of spermatozoa and non-sperm cells in the ejaculate to be calculated.^[21]

The sample should be collected after a minimum of 2 days and a maximum of 7 days of sexual abstinence. If additional samples are required, the number of days of sexual abstinence should be as constant as possible at each visit.^[22]

1.7.1 Methods of measuring seminal fluid ejaculated volume

- The volume is best measured by weighing the sample in the container (clean, wide-mouthed container made of glass or plastic, from a batch that has been confirmed to be non-toxic for spermatozoa) in which it is collected. Collect the sample in a pre-weighed, disposable container. Weigh the container with semen in it. Subtract the weight of the container. Calculate the volume from the sample weight, assuming the density of semen to be 1 g/ml. Use a permanent marker pen on the vessel itself or on a label. If a label is used for recording the weight, it should be attached before the empty container is weighed.^[20]
- Alternatively, the volume can be measured directly. Collect the sample directly into a modified graduated glass measuring cylinder with a wide mouth. These can be obtained commercially. Read the volume directly from the graduations (0.1 ml accuracy).
- Measuring volume by aspirating the sample from the specimen container into a pipette or syringe, or decanting it into a measuring cylinder, is not recommended, because not all the sample will be retrieved and the volume will therefore be underestimated. The volume lost can be between 0.3 and 0.9 ml.^[21]

The aims of the study

This study aims to find if there is relation between seminal fluid volume and grades of varicocele by

ultrasound and to estimate if there is any effect on fertility through this seminal fluid parameter.

2.1 Study setting and Design

This study conducted at Radiology department at Al - Shahid Ghazi Al -Hariri Teaching Hospital at out-patient clinic during the period between September 2020 to the March 2021.

This study was prospective case control study included 100 men with subfertility with average age 25-40 years. 50 sub-fertile men with varicocele (case group) and 50 sub-fertile men without varicocele (control group).

2.2 Selection of the study samples

100 suspected subfertile men referred from outpatient clinic was included in this study. Verbal and informed consent was taken from the study population.

Exclusion Criteria

patients with any pathology which could affect fertility, such as infection, cryptorchidism, prior surgery, obstruction of the ejaculatory duct or congenital bilateral absence of the vas deferens (CBAVD), a condition in which the seminal vesicles are also poorly developed and age < 25 or >40 years were also excluded.

2.3 Data Collection

A preformed questionnaire had been used to get information from studied population (Appendix 1). High-frequency ultrasonography with color Doppler imaging was performed using 5- 7.5-MHz transducers (Philips HD11 and Samsung HS40). The pampiniform plexus was scanned in supine and standing positions before and during a Valsalva maneuver.

A varicocele was diagnosed when 2 or more tortuous dilated veins were identified, reflux flow persisting for more than 2 second based on Doppler color change, and increased retrograde flow in the pampiniform plexus before or during a Valsalva maneuver in the supine or standing position.our study depend on the flowing grading system.^[11]

Table 1: Grades Of Varicocele By Ultrasound.

Grade	Relaxed state	During Valsalva
Normal	2.2mm	2.7mm
Small varicocele	2.5-4.0 mm	↑ by 1.0mm
Moderate varicocele	4.0-5.0mm	↑ by 1.2-1.5mm
Large varicocele	> 5.0mm	↑ by > 1.5mm

Varicoceles diagnosed when 1 or more veins had a maximal diameter of more than 2.5 mm.

The volume measured directly after Collection the sample directly into a modified graduated glass or plastic measuring cylinder with a wide mouth. The volume

directly seen from the graduations (0.1 ml accuracy). The lower reference limit for semen volume is 1.5 ml.^[21]

2.4 Data Analysis

The data were entered in computer using Microsoft excel program 2010 and analysis was done using the software (MINITAB) version 10 for windows. statistical Independent t-test for two sample and Chi square test was used.

P – Value regarded significant when it was < 0.05

THE RESULTS

A total of 100 males were contributed in this study, 50 of them had varicocele (cases), and 50 not suffered from

varicose (control). The mean age of cases was (30.76 $\pm$ 5.62 year), which was not significantly different from mean age of control group (31.92 $\pm$ 7.59 year), p value=0.327, table 2.

The mean level of seminal fluid volume in cases group was (2.86 $\pm$ 0.87) which is not significant with that of control group,(3 $\pm$ 0.70) P value=0.802, table1.

Table 2: Differences between seminal fluid volume and age according to studied group by using independent 2 samples t test.

	Studied sample	N	Mean	Std. Deviation	P value
Age	cases	50	30.76	5.62	0.327
	control	50	31.92	7.59	
Seminal fluid volume	cases	50	2.86	0.871	0.802
	control	50	3.00	0.707	

As shown in table 3 no significant association were noticed between age similar in both study groups (p value =0.32).

Table 3: Association between age groups and groups of study sample by using Chi square test.

Age/years	case		control		P value
	no	%	no	%	
25-30 years	22	47.8	24	52.2	0.32
31-35 years	24	57.1	18	42.9	
36-40 years	4	33.3	8	66.7	
Total	50	50	50	50	

According to the amount of seminal fluid, there were 5 participant's presented with low volume (<1.5 m), 3 of them were cases and 2 were control. There was no

significant association between seminal fluid volume and study group, table4.

Table 4: Association between studied group and amount of seminal fluid:

Seminal fluid volume		Study group		Total
		Case	Control	
Normal seminal fluid volume	No.	47	48	95
	%	49.5	50.5	100.0
Low seminal fluid volume	No.	3	2	5
	%	60.0	40.0	100.0
Total	No.	50	50	100
	%	50.0	50.0	100.0
P value		0.501		

Table 4 shows that there were no significant association between amount of patients' seminal fluid and right and left side grade of varicose, (P=0.88, P=0.17 respectively).

There were 36 patients presented with unilateral varicose, two of them with low seminal fluid volume and there were 14 patients with bilateral varicose, one of them with low volume. No significant association

between seminal fluid volume and affected side with varicocele. Table 5.

Table 5: Association between amount of patients' seminal fluid and other variables (right and left side grade of varicose and affected side with varicose).

		Seminal fluid						P value
		Normal seminal fluid volume		Low seminal fluid volume		Total		
		No.	%	No.	%	No.	%	
Right side grade of varicose	no varicose	34	94.4	2	5.6	36	100.0	0.88
	Grade I	11	91.7	1	8.3	12	100.0	
	Grade II	2	100.0	0	0.0	2	100.0	
	Grade III	0	0.0	0	0.0	0	0.0	
Left side grade of varicose	no varicose	0	0.0	0	0.0	0	0.0	0.17
	Grade I	16	100.0	0	0.0	16	100.0	
	Grade II	21	95.5	1	4.5	22	100.0	
	Grade III	10	83.3	2	16.7	12	100.0	
Affected side with varicose	Unilateral varicose	34	94.4	2	5.6	36	100.0	0.83
	Bilateral varicose	13	92.9	1	7.1	14	100.0	

DISCUSSION

Varicocele which is an abnormal dilatation, compressibility, and tortuosity of the pampiniform plexus, resulting in retrograde flow into the internal spermatic vein and it was found that it play rules in the male causes of infertility The prevalence of varicocele is approximately 15% to 20% in the male population and 30% to 40% in sub-fertile men.

After collecting the data from the patient in Radiological Department of Al- Shahid Ghazi Al -Hariri Teaching Hospital the study shows that there is no significant important relation between the age in case of varicocele and seminal fluid low volume (as shown in result table 1).

Vivas-Acevedo et al study that include 363case was got varicocele (cases), and 190 not suffered from varicocele (control) .The mean age of cases was (28.3 $\pm$ 8.8 year), which was not significantly different from mean age of control group (32.3 $\pm$ 7.5 year), p value=0.327.^[23]

The mean level of seminal fluid volume in cases group was (3.5 $\pm$ 1.6) which is not significant with that of control group,(3.7 $\pm$ 1) P value=0.723.

The study done by Blumer et al shows that the mean age of patient with varicocele 34.0 $\pm$ 4.7 (32.2, 35.7), there seminal fluid volume 3.5 $\pm$ 1.6 (2.9, 4.1) and the P value for this group was about 0.307 and there control group (patient without varicocele)there mean age 35.1 $\pm$ 4.2 (33.6, 36.6), the seminal fluid volume of about 3.3 $\pm$ 1.4 (2.8, 3.8) and the P value of about 0.569 so this mean no significant association between age of patient, present or absent of varicocele and seminal fluid volume.^[24]

Depending on the statistic calibration that have been done in our study the result shows no significant relation between the grades of varicocele and seminal fluid volume and the P value that appear about 0.88.

This because most of the seminal fluid volume not produced from the testicles and its was mostly produced from the prostatic gland and bulbourethral glands and a little amount of its volume was produces from epididymous and testicles. Semen is formed during ejaculation from a condensed suspension of spermatozoa, which is retained in the paired epididymides and then combined with and diluted by fluid secretions from the accessory sex organs. It comes in a variety of boluses. When comparing pre- and post-vasectomy semen volumes, it's clear that accessory organ secretions account for roughly 90% of semen volume.^[21]

Agarwal et al mention in his study that the result of six studies reported data on semen volume including 936 men (605 with varicocele and 331 controls). Mean semen volume in patients and controls ranged from 2.6 to 3.3 ml and 2.6 to 3.7 ml, respectively. in the same study shows another five studies reported that semen volume was not statistically different between men with varicocele and controls. Heterogeneity was high and REM(random effect model), therefore, provided the actual representation of the data. Overall, random effect model indicated that semen volume was not significantly affected by varicocele (mean difference: -0.23 ml; 95% CI: -0.64, 0.17; $P = 0.26$). To analyze the potential causes of the heterogeneity, a subgroup analysis was conducted to assess the effect of WHO manual editions with regards to semen volume. Performing separate analyses according to the WHO manual edition significantly reduced heterogeneity estimates, but the observed pooled effect size was not materially affected.^[25]

Other study by Will et al show, 19.5% of patients had at least one abnormal semen parameter. The leading abnormality was asthenospermia, followed by oligospermia and teratospermia. Therefore, semen analysis would be warranted as a screening test for semen abnormalities regardless of the chief complaint in young varicocele patients. Seminal fluid volume of these patient of about 3.3 $\pm$ 1.4 ml, P value of about 0.569 which mean no significant association.^[26]

Costabile et al show in his study that Participants in the experimental group had significantly lower baseline mean sperm concentration and sperm motility and higher mean testicular volume differences than the participants in the comparison group (all with $P < .0001$); there was no significant group difference in baseline mean semen volume.^[27]

The study by Yamamoto shows that Varicocele ligation resulted in significantly increased mean sperm concentration and motility and significantly decreased mean testicular volumes 3 months and 6 months after surgery (all with $P < .0001$). There was no significant change in semen volume. Sperm concentration and sperm motility reached near-normal levels following surgery, but patients continued to have significantly larger mean testicular volume differences than those of the comparison group. Mean right and left testicular volume difference was significantly correlated with mean sperm motility 6 months postoperatively (Pearson $r = -0.365$; $P = .009$), but not significantly correlated with sperm concentration or semen volume. The partners of 24 patients (48%) were pregnant 6 months postoperatively.^[28]

Comparison of pre- and post-vasectomy semen volumes by Amidu et al study reveals that about 90% of semen volume is made up of secretions from the accessory organs, mainly the prostate and seminal vesicles, with minor contributions from the bulbourethral (Cowper's) glands and epididymides.^[20]

Limitations

Our locality labs do the measurement of seminal fluid volume by using container of side wall ml measurement or they used another method of measurement by using syringe or pipet after 1 hour of liquefaction but the last method gives less accurate measurement (low result by about 0.3-0.9 ml) and this will give false result about the accurate seminal fluid volume.

We also found difficulties in collecting seminal fluid data from the patient because they only do the Doppler ultrasound and not return for our radiological unit to complete our study.

CONCLUSION

This study investigated the relationship between varicocele and seminal fluid volume among 100 male participants, including 50 patients with varicocele as the case group and 50 participants without varicocele as the control group. The findings showed that there was no statistically significant difference in age between the two groups. The mean age was 30.76 ± 5.62 years among cases and 31.92 ± 7.59 years among controls ($p = 0.327$), indicating that the groups were comparable in terms of age.

The mean seminal fluid volume was 2.86 ± 0.87 mL in patients with varicocele compared with 3.00 ± 0.70 mL

in the control group. This difference was not statistically significant ($p = 0.802$). Similarly, when seminal fluid volume was categorized as normal or low, no significant association was found between seminal fluid volume and the presence of varicocele ($p = 0.501$). Only five participants had low seminal fluid volume, including three cases and two controls.

Furthermore, no significant association was observed between seminal fluid volume and the grade of varicocele on either the right or left side. There was also no significant relationship between seminal fluid volume and the affected side, whether unilateral or bilateral. These findings indicate that neither the presence nor the severity or laterality of varicocele was significantly associated with reduced seminal fluid volume in the studied population.

The results are consistent with previous studies showing that varicocele may affect certain semen parameters, particularly sperm concentration, motility, and morphology, without necessarily reducing total seminal fluid volume. This may be explained by the fact that most semen volume is produced by the accessory reproductive glands, mainly the seminal vesicles and prostate, rather than the testes.

In conclusion, this study found no significant association between varicocele and seminal fluid volume. Seminal fluid volume alone may therefore not be a reliable indicator of the reproductive effects of varicocele. Further studies with larger sample sizes, standardized measurement methods, and assessment of additional semen parameters are recommended.

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