

The Role of Anogenital Distance Measurement in the Prediction of The Efficacy of Varicocelectomy in Primary Male Infertility

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Abstract

Background: Anogenital distance is a marker of genital development and adult testicular function. It is currently difficult to assess which patients are most likely to benefit from varicocelectomy. The present study showed that men with a longer AGD had a higher likelihood of improvement after varicocelectomy.

Aim of Study: Is to evaluate the role of Anogenital Distance (AGD) measurement in the prediction of the efficacy of varicocelectomy on the improvement of semen parameters in primary male infertility.

Material and Methods:

- A prospective study included sixty male patients presented with primary infertility and clinical evident primary varicocele with altered semen parameters.
- All patients underwent measuring the distance from the posterior aspect of the scrotum to the anal verge in millimeters using a digital caliper.
- All patients underwent varicocelectomy (subinguinal approach) by the same experienced surgeon using intraoperative Doppler and magnification loupe.
- All patients were followed-up with semen analysis after 6 months of varicocele repair.

Results:

- Overall, 76.7% of our patients (46 patients) revealed improvement in their semen parameters after 6 months of varicocelectomy, men with longer AGD had better improvement in their semen parameters after surgery than men with shorter AGD, 30 patients (88.2%) with AGD \geq 30mm vs. 16 patients (61.5%) with AGD $<$ 30mm.

- In patients with AGD $>$ 30mm. The sperm concentration increased from 13.5 to 18.8 million sperm per ml (p -value $<$ 0.001), the progressive motility (A + B) increased from 27.9% to 39.5% (p -value $<$ 0.001), the mean abnormal forms decreased from 57.2% to 36.2% (p -value $<$ 0.001). While in the other group with AGD $<$ 30mm there was no statistical differences in different semen parameters post-operatively.

Conclusions: AGD may provide a novel metric to assess intrinsic testicular function and predict efficacy of varicocele repair.

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Introduction

A **VARICOCELE** is an abnormal dilatation and tortuosity of veins of the pampiniform plexus that drain the testis. Almost all varicoceles are detected after puberty and there is a prevalence of about 11-15% among adult men [1,2].

Varicoceles are considered to be the commonest correctable cause of male infertility [3]. Their incidence among infertile men is 20-40% about three times greater than in the general population. The mechanism by which varicoceles affect testicular function remains unclear. The most commonly accepted hypothesis is that varicoceles result in an increase in testicular temperature that suppresses spermatogenesis [4,5].

Varicoceles as a cause of male infertility have been the subject of debate. There are a number of issues that fuel such debate. Many men with varicoceles are fertile. On the other hand, since varicoceles are so common, a varicocele may be coincidentally-but not causatively-present in an infertile man. Thus, not all varicoceles cause infertility and not all need correction. There have been severely discordant results in the literature regarding the benefits of varicocele surgery in men with infertility. Finally, there is no universally accepted theory on the mechanism of action of varicoceles in inhibiting fertility.

In clinical practice, varicocele-related testicular damage may show persistent abnormalities of semen analysis parameters including concentration, motility, and/or morphology [6-10]. While many investigators have reported that treatment of varicocele improves semen parameters, pregnancy rates, and intrauterine insemination pregnancy and

birth rates, a sub-set of men will not benefit after varicocelectomy. Indeed, it is estimated that semen parameters will improve in 60% of patients after varicocele repair [11].

A sexually dimorphic measure of genital development under hormonal influence, Anogenital Distance (AGD) was initially studied in animals [12-14]. Investigators have also used AGD to show that agents that disrupt androgen signaling in male animal models can lead to abnormal genital lengths and even altered testicular function as measured by testosterone and sperm production [15-18]. Human studies have also linked AGD to adult testicular function, as assessed by testosterone and sperm production [19-21]. However, the clinical utility of such measurements remains unclear.

It may provide a non-invasive method to assess male reproductive potential and testicular function, it may predict men with better inherent testicular function whose impaired semen parameters due to varicocele may be more likely to improve after varicocelectomy. So, we studied the impact of AGD length on improvement of semen parameters after varicocele repair.

Patients and Methods

After approval from Institutional Review Board, we conducted a prospective study in Urology Department Faculty of Medicine, Tanta University, EGYPT from February 2017 to January 2018.

Our study included 60 male patients who presented with primary infertility and clinically evident primary varicocele with altered semen parameters. Men with azoospermia and secondary varicocele were excluded. Also, men with a history of orchidectomy, testicular torsion, previous malignancy, prior testosterone use, or prior chemotherapy exposure were excluded.

All patients underwent measuring the distance from the posterior aspect of the scrotum to the anal verge in millimeters using a digital caliper in the supine, frog-leg position with the legs abducted, allowing the soles of the feet to meet. And evaluation of AGD was based on normal value of 3cm determined by Eisenberg et al., 2012 [21].

All patients underwent varicocelectomy (subinguinal approach) by the same experienced surgeon.

Informed consent was taken from the patient with explanation of the potential complications of the procedure.

Patient evaluation:

All the patients were subjected to the following:

- 1- History taking including present and past history (e.g. history of infertility, sexual history, surgical history, and medical history).
- 2- General examination: Including: Age, weight and height and BMI.
- 3- Local examination including:
 - *Testicular size and consistency*: Testis size was estimated by comparison with a Prader orchimeter modified for use in adults.
 - *Examination of varicocele*: According to the clinical staging system of Dubin and Almela (1970) varicocele is classified into: Grade III varicocele, which is visible before palpation. Grade II varicocele, is detected on palpation, but with no need for a Valsalva maneuver. Grade I varicocele is detected on palpation with the Valsalva maneuver only.
- 4- Genital measurement (Anogenital distance):

The distance from the posterior aspect of the scrotum to the anal verge was measured in millimeters using a digital caliper (iGaging, Ez Cal IP54 Digital caliper) in the supine, frog-leg position with the legs abducted, allowing the soles of the feet to meet.
- 5- *Imaging studies*: Scrotal Doppler ultrasonography to confirm diagnosis of varicocele and measure testicular size.
- 6- *Laboratory investigations*:
 - a- CBC, liver, function, kidney function, fasting, postprandial blood sugar and Prothrombin activity.
 - b- *Semen analysis*:
 - 1- Men were given clear instructions about how to produce a sample. Samples were produced after a period of abstinence of 2-7 days.
 - 2- Pre-operative semen analysis: 2 samples of Computer-Assisted Semen Analysis (CASA) are collected from our patients with 2 weeks at least in between.
 - c- *Hormonal analysis*: Testosterone (free and total), FSH, LH and Prolactin.

Follow-up:

All patients were followed-up with semen analysis after 6 months of varicocele repair.

Statistical analysis of the data:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0.

(Armonk, NY: IBM Corp). The Kolmogorov-Smirnov, Shapiro and D'agstino tests were used to verify the normality of distribution of variables, Comparisons between groups for categorical variables were assessed using Chi-square test (Fisher or Monte Carlo). Student *t*-test was used to compare two groups for normally distributed quantitative variables while Mann Whitney test was used to compare between two groups for abnormally distributed quantitative variables. Paired *t*-test was assessed for comparison between two periods. While Wilcoxon signed ranks test was assessed for comparison between different periods for abnormally distributed quantitative variables. Signif-

icance of the obtained results was judged at the 5% level.

Results

Pre-operative data: Regarding the demographic and anthropomorphic data of our studied cohort, we reported no statistical significant difference between patients with AGD ≥ 30 mm and patients with AGD < 30 mm. Eight of 60 men underwent a unilateral varicocelectomy and 52 men underwent a bilateral procedure with no significant difference in between patients according laterality and grading of varicocele (Table 1).

Table (1): Comparison between the two studied groups according to different parameters.

	Total (n=60)	AGD		Test of sig.	p
		≥ 30 mm (n=34)	< 30 mm (n=26)		
Age (years):					
≤ 30	24 (40%)	16 (47.1%)	8 (30.8%)	$\chi^2 = 1.629$ $t = 0.785$	0.202
> 30	36 (60%)	18 (52.9%)	18 (69.2%)		
Median (Min. Max.)	32 (26-39)	32 (26-38)	33 (27-39)		
Mean \pm SD	31.8 \pm 3.3	31.5 \pm 3.4	32.2 \pm 3.3		
Height (cm):					
Median (Min. Max.)	174 (160-180)	174 (160-180)	174 (164-180)	$t = 0.669$	0.506
Mean \pm SD	172.7 \pm 5.5	173.1 \pm 5.7	172.2 \pm 5.3		
Weight (kg):					
Median (Min. Max.)	87.5 (74-105)	85 (74-105)	90 (79-103)	$t = 1.629$	0.109
Mean \pm SD	88.3 \pm 8.6	86.7 \pm 9.4	90.3 \pm 7.1		
BMI (kg/m²):					
Median (Min. Max.)	30.1 (22.8-34)	28.7 (22.8-32.4)	30.4 (28.1-34)	U=280.0*	0.016*
Mean \pm SD	29.6 \pm 2.2	28.9 \pm 2.5	30.4 \pm 1.5		
Laterality:					
Unilateral	8 (13.3%)	6 (17.6%)	2 (7.7%)	$\chi^2 = 1.263$	0.446
Bilateral	52 (86.7%)	28 (82.4%)	24 (92.3%)		
Grade:					
Right:					
I	6 (11.5%)	2 (7.1%)	4 (16.7%)	$\chi^2 = 1.360$	0.492
II	38 (73.1%)	22 (78.6%)	16 (66.7%)		
III	8 (15.4%)	4 (14.3%)	4 (16.7%)		
Left:					
II	28 (46.7%)	16 (47.1%)	12 (46.2%)	$\chi^2 = 0.005$	0.944
III	32 (53.3%)	18 (52.9%)	14 (53.8%)		
Lt Testicular size by us (cm³):					
Median (Min. Max.)	11.5 (8-20)	11 (9-20)	12 (8-18)	$t = 1.285$	0.504
Mean \pm SD	13 \pm 3.8	13.5 \pm 4.4	12.3 \pm 3		
Total testosterone (ng/ml):					
Median (Min. Max.)	6.5 (2.8-10.3)	6.8 (2.8-10.3)	6.4 (3.9-9.8)	$t = 0.044$	0.965
Mean \pm SD	6.4 \pm 2.2	6.4 \pm 2.5	6.4 \pm 1.7		
Free testosterone (pg/ml):					
Median (Min. Max.)	155 (50-290)	160 (70-280)	150 (50-290)	$t = 0.617$	0.540
Mean \pm SD	161.7 \pm 74.2	156.5 \pm 69.9	168.5 \pm 80.4		
FSH (mIU/ml):					
Median (Min. Max.)	4.9 (1.7-10.8)	4.2 (1.9-10.2)	6.7 (1.7-10.8)	U=376.0	0.324
Mean \pm SD	5.6 \pm 2.9	5.2 \pm 2.6	6.2 \pm 3.1		
LH (mIU/ml):					
Median (Min. Max.)	3.8 (0.7-8.9)	3.6 (0.9-7.6)	4.3 (0.7-8.9)	U=352.0	0.179
Mean \pm SD	4 \pm 2.1	3.7 \pm 2	4.4 \pm 2.1		
Prolactin (ng/dl):					
Median (Min. Max.)	11.3 (2.6-16.8)	12.3 (2.6-16.8)	11.3 (2.6-16.8)	U=406.0	0.590
Mean \pm SD	10.2 \pm 4.9	10 \pm 5.1	10.2 \pm 4.9		

The number of patients with AGD <30mm was 26 patients, while the number of patients with AGD ≥30mm was 34 patient with mean of 3.4 ± 0.8mm.

All patients included in this study underwent hormonal profile assessment in form of total testosterone, free testosterone, FSH, LH and prolactin. There was no statistical significant difference between both groups.

There was no statistical significant difference between both groups regarding the pre-operative semen analysis (Table 2).

Post-operative data: Overall, 76.7% of our patients (46 patients) revealed improvement in their semen parameters after 6 months of varicoce-

lectomy, men with longer AGD had better improvement in their semen parameters after surgery than men with shorter AGD, 30 patients (88.2%) with AGD ≥30mm vs. 16 patients (61.5%) with AGD <30mm (Table 3).

Our data showed that the mean sperm concentration increased from 13.5 to 18.8 million sperm per ml (p -value <0.001), the mean progressive motility (A + B) increased from 27.9% to 39.5% (p -value <0.001), the mean abnormal forms decreased from 57.2% to 36.2% (p -value <0.001) in patients with AGD ≥30mm (Table 4).

There was no significant difference in improvement of semen volume in patients with AGD ≥30mm and patients with AGD <30mm (Table 4).

Table (2): The pre-operative semen analysis of cohort.

Semen analysis	Total (n=60)	AGD length		Test of sig.	<i>p</i>
		≥30mm (n=34)	<30mm (n=26)		
<i>Volume:</i>					
Median (Min.-Max.)	2.5 (1-4.5)	2.5 (1-4.5)	2.5 (1-4)	<i>t</i> =0.037	0.971
Mean ± SD	2.5±1	2.5±1	2.5±0.9		
<i>Concentration:</i>					
Median (Min.-Max.)	9.5 (0.4-45)	9 (0.4-45)	11 (4.9-40)	U=354.0	0.189
Mean ± SD	13.6±11.3	13.5±12.9	13.6±9.1		
<i>Motility:</i>					
• A:					
Median (Min.-Max.)	11 (2-35)	12 (2-33.5)	10 (5-35)	U=390.0	0.433
Mean ± SD	13.8±8.2	12.6±7.3	15.4±9.2		
• A + B:					
Median (Min.-Max.)	28 (10-50)	25 (10-47)	29 (20-50)	U=288.0*	0.020*
Mean ± SD	29.8±10.2	27.9±10.2	32.2±9.9		
<i>Abnormal forms:</i>					
Median (Min.-Max.)	60 (20-96)	60 (20-96)	60 (30-90)	<i>t</i> =0.511	0.611
Mean ± SD	58.4±22.7	57.2±26	60.1±17.9		

Table (3): The number of the patients who had improvement in different semen parameters.

Improvement	Total (n=60)	% improvement AGD length		Test of sig.	<i>p</i>
		≥30mm (n=34)	<30mm (n=26)		
Volume	28 (46.7%)	14 (41.2%)	14 (53.8%)	$\chi^2=0.950$	0.330
Concentration	40 (66.7%)	28 (82.4%)	12 (46.2%)	$\chi^2=8.688^*$	0.003 *
<i>Motility:</i>					
A	36 (60%)	28 (82.4%)	8 (30.8%)	$\chi^2=16.335^*$	<0.001*
A + B	38 (63.3%)	26 (76.5%)	12 (46.2%)	$\chi^2=5.831^*$	0.016*
Abnormal forms	34 (56.7%)	28 (82.4%)	6 (23.1%)	$\chi^2=21.082^*$	<0.001*
Overall improvement	46 (76.7%)	30 (88.2%)	16 (61.5%)	$\chi^2=5.870^*$	0.015*

Table (4): Comparison between pre-operative and 6 months post-operative semen analysis in our studied patients regarding AGD.

Semen analysis	AGD (mm)			
	≥30 (n=34)		<30 (n=26)	
	Baseline	After 3 month	Baseline	After 3 month
<i>Volume:</i>				
Median (Min.-Max.)	2.5 (1-4.5)	3 (1-4)	2.5 (1-4)	2.5 (1-4)
Mean ± SD	2.5±1	2.7±0.9	2.5±0.9	2.7±0.9
t_p		0.059		0.008*
<i>Concentration:</i>				
Median (Min.-Max.)	9 (0.4-45)	18 (2-40)	11 (4.9-40)	11 (4.5-35)
Mean ± SD	13.5±12.9	18.8±11.7	13.6±9.1	14.3±8
Z_p		<0.001*		0.218
<i>Motility:</i>				
• A:				
Median (Min.-Max.)	12 (2-33.5)	19 (9-40)	10 (5-35)	10 (1-30)
Mean ± SD	12.6±7.3	20.4±8.6	15.4±9.2	15.5±10.6
Z_p		<0.001*		0.314
• A + B:				
Median (Min.-Max.)	25 (10-47)	40 (20-55)	29 (20-50)	30 (20-60)
Mean ± SD	27.9±10.2	39.5±10.4	32.2±9.9	32.6±12.3
Z_p		<0.001*		0.728
<i>Abnormal forms:</i>				
Min.-Max.	60 (20-96)	40 (10-60)	60 (30-90)	60 (15-90)
Mean ± SD	57.2±26	36.2±17.1	60.1±17.9	58.3±22.2
t_p		<0.001*		0.764

Discussion

Varicocele is traditionally indicated in subfertile men with varicoceles who have oligospermia, asthenospermia, teratospermia or combinations of these features. Several investigators have demonstrated improvement in semen quality in 51-74% of patients and an increased pregnancy rate of 24-71 % after varicocele treatment. However, some authors reported no beneficial effect of varicocele treatment on semen quality and pregnancy rates [22].

Marmar et al., [23] performed a systematic review of pregnancy outcomes after varicocele treatment but included only studies of men with palpable varicoceles and abnormal semen parameters. The authors found significantly increased pregnancy rate after varicocele treatment.

Investigators have used AGD to show that agents that disrupt androgen signaling in male animal models can lead to abnormal genital lengths and even altered testicular function as measured by testosterone and sperm production [15-18].

Indeed, Hsieh et al. showed shorter AGDs in boys with genital anomalies, i.e. hypospadias and cryptorchidism, establishing a link between normal genital development and AGD in humans [13].

Mendiola J et al., 2015 [24] assessed AGD measures in men attending infertility services in

Europe to examine the relationship between both variants of AGD which include AGDAS (measured from the posterior base, first fold of the scrotum, to the center of the anus) and AGDAP (measured from the cephalad insertion of the penis to the center of the anus). and semen parameters in male seeking fertility treatment. They observed significant positive association between AGDAS measures and sperm concentration, total sperm count and total sperm motile count. These findings correlate with our results that showed a positive association between AGDAS measures and sperm concentration, total sperm count.

Eisenberg ML et al., 2011 [19] demonstrated in a cohort of U.S. adult men evaluated in an andrology practice that anogenital distance was positively correlated with a man's fertility potential as assessed by sperm production. Also, they reported that AGD was significantly correlated with sperm density and total motile sperm count. They were adjusted for demographic and reproductive variables, for each 1 cm increase in a man's AGD, the sperm density increases by 4.3 million sperm per mL and the total motile sperm count increases by 6.0 million sperm.

Mendiola J et al., 2011 [20] showed that AGD is also related to fatherhood, fertility and adult sperm production. Thus, AGD may reflect the spermatogenic potential of the testis. In their study

AGD was measured in adult men and they examined the relationships between AGD measures and sperm parameters. They observed significant positive associations between AGD and sperm concentration, motility, morphology, total sperm count, and total motile count. The associations they observed between these sperm parameters and AGD were stronger than those for most covariates known to be associated with semen quality. Moreover, a man with an AGD below the median was 7.3 times as likely to have a sperm concentration in the subfertile range in comparison to a man with an AGD above the median.

These results from Mendiola J et al., correlate with our results as we found a significant difference in the percentage of improvement of semen concentration, motility and abnormal forms in patients with AGD \geq 30mm in comparison to men with shorter AGD 6 months after surgery.

Eisenberg ML et al., 2012 [25] showed that a longer AGD in infertile men suggest improvement of varicocelectomy outcomes. Surprisingly it was found that even men with shorter AGDs still benefited from repair, as 62% of them showed an improved total motile sperm count after repair.

This is also correlates with our current study as 76.7% of our patients revealed improvement in the semen parameters after 6 months of surgery, 88.2% of them with AGD \geq 30mm, 61.5% with AGD $<$ 30mm, so a greater proportion of men with longer AGD show improvement in semen parameters after varicocelectomy than men with shorter AGD.

In contrast to our study, Parra et al., showed that AGD measures were not associated with any semen parameters or any of the reproductive hormone levels in Caucasian young men from southern Spain [26]. But it is not truly against our study as their study was not done among infertile men with abnormal semen parameters and the majority of their study was among men with AGD more than 30mm. in addition, they did not perform varicocelectomy to compare the association between AGD and the efficacy on semen parameter after varicocelectomy.

Certain limitations warrant mention in our study. The sample size was relatively small and limited in ethnicity, which may have limited our ability to detect associations between AGD measurement and semen quality parameters, but we found significant associations. In addition, only one pre-operative semen sample was collected to evaluate semen parameters. Nevertheless, a number of stud-

ies have reported that one sample is enough to assess semen quality in epidemiological studies [27].

In addition, only men referred to and evaluated in our clinic were eligible for enrollment; therefore, it is possible that our patient population does not represent all men.

To our knowledge, our present study represents the first clinical analysis of AGD for Egyptian infertile men with varicocele, suggesting that AGD may provide prognostic information in treatment outcomes of varicocelectomy.

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دور قياس المسافة الشرجية التناسلية في التنبؤ بمدى فاعلية استئصال دوالي الخصية في العقم الذكوري الأولي

دوالي الخصية هي عبارة عن تعرج وتمدد غير طبيعي في الأوردة الدموية التي تصب بها الخصيتين وتحدث في الحبل المنوي ومن الممكن الكشف عنها بالفحص الإكلينيكي وغالباً ما تحدث دوالي الخصية بعد سن البلوغ مع نسبة حدوث من ١٠:١٥٪ في الرجال البالغين.

تعتبر دوالي الخصية من أشهر أسباب العقم التي يمكن علاجها ونسبة حدوثها في الرجال العقيمة تكاد تقترب من ثلاثة أضعاف حدوث الدوالي في الرجال الطبيعيين.

تعتبر دوالي الخصية كسبب للعقم عند الرجال من أكثر المواضيع جدلاً فهناك بعض الرجال ذى الدوالي لا يعانون من العقم وفي المقابل ونظراً لانتشار نسبة حدوث دوالي الخصية فهناك احتمالية حدوث العقم مع وجود دوالي الخصية.

في الحياة العملية توجد كثير من التقارير التي تؤكد علاقة دوالي الخصية بتدبير الخصية والتأثير على الحيوانات المنوية من حيث العدد والحركة والتركيز والشكل كما أثبتت الفحوصات أيضاً أن علاج الدوالي يحسن كثيراً من تحليل السائل المنوي وارتفاع نسبة حدوث حمل ولكن في المقابل يوجد مجموعة من المرضى الذين لا يستفيدون من علاج الدوالي.

يعتبر قياس الشرجية التناسلية وهي المسافة من الأعضاء التناسلية لفتحة الشرج من القياسات التي تم استخدامها وإجراء تجارب عليها في الحيوانات ويوجد دراسة حديثة على الإنسان تثبت أن قياس الشرجية التناسلية لها علاقة بوظيفة الخصية من حيث إنتاج الحيوانات المنوية ونسبة هرمونات الذكورة.

يمثل قياس الشرجية التناسلية وسيلة آمنة لقياس قدرة الجهاز التناسلي للرجال من حيث القدرة الجنسية ومن حيث كفاءة الخصية ومن الممكن عن طريق هذا الفحص توقع المرضى الذين سيحدث لهم تحسن بعد علاج دوالي الخصية مع احتمالية تحسن تحليل السائل المنوي طبقاً لقياس الشرجية التناسلية.