International Journal of Andrology

The relationship between anogenital distance and the etiology of azoospermia in adult men

Journal:	*International Journal of Andrology*
Manuscript ID:	IJA-2011-0352
Manuscript Type:	Original Article
Date Submitted by the Author:	27-Dec-2011
Complete List of Authors:	Eisenberg, Michael; Stanford University School of Medicine, Urology Shy, Michael; Baylor College of Medicine, Urology Walters, Rustin Lisphultz, Larry; Baylor College of Medicine, Urology
Key Words:	genitalia, perineum, azoospermia, humans

The relationship between anogenital distance and the etiology of azoospermia in adult men

Michael L. Eisenberg, MD,[1] Michael Shy, MD, PhD,[2] R. Chanc Walters, MD,[2] Larry I. Lipshultz, MD[2]

[1] Department of Urology, Stanford University School of Medicine, Palo Alto, CA

[2] Division of Male Reproductive Medicine and Surgery, Scott Department of Urology, Baylor College of Medicine, Houston, TX

Keywords: genitalia, perineum, azoospermia, humans

Corresponding Author:
Michael L. Eisenberg, M.D.
Department of Urology
Stanford University School of Medicine
300 Pasteur Drive
Stanford, CA 94305-5118
Phone: 650-723-4537
Fax: 650-498-5346
Email: eisenberg@stanford.edu

Abstract:

Anogenital distance (AGD) is a marker for endocrine disruption in animal studies in which decreased AGD has been associated with testicular dysfunction. The objective of the study was to investigate whether anogenital distance could distinguish men with obstructive (OA) from those with nonobstructive azoospermia (NOA). To accomplish this, azoospermic men were recruited and evaluated at a men's reproductive health clinic in Houston, Texas. Anogenital distance (the distance from the posterior aspect of the scrotum to the anal verge) and penile length (PL) were measured using digital calipers. Testis size was estimated by physical examination. Logistic regression was used to compare AGD lengths in men with OA and men with NOA. A total of 69 OA men (mean age: 44.2 ± 9.2) and 29 NOA men (mean age: 32.8 ± 4.8) were recruited. The NOA men possessed significantly shorter mean AGD than the men with OA (AGD: 36.3 vs 41.9 mm, p=0.01). An AGD of less than 30 mm, had a 91% specificity in accurately classifying NOA. Moreover, after adjustment for age, race, and BMI, an AGD of less 30 mm yielded a significantly increased odds of NOA compared to OA (OR 5.56, 95 % CI: 1.01, 30.69). In summary, AGD may provide a novel metric for assessing testicular function in men and in distinguishing OA from NOA.

Introduction

A sexually dimorphic measure of genital development under hormonal influence, AGD was initially used to sex animals.(Greenham & Greenham, 1977; Hsieh, *et al.*, 2008; Marois, 1968) More recently, human studies have also shown that boys have longer perineal lengths than girls.(Salazar-Martinez, *et al.*, 2004; Sathyanarayana, *et al.*, 2010; Thankamony, *et al.*, 2009; Torres-Sanchez, *et al.*, 2008) Investigators have also used AGD to show that agents which disrupt androgen signaling in animal models can lead to abnormal genital lengths and even altered testicular function as measured by testosterone and sperm production.(Cowin, *et al.*, 2010; Foster, *et al.*, 2001; Martino-Andrade, *et al.*, 2009; Scott, *et al.*, 2008) Human studies have also linked adult testicular function, as assessed by sperm and testosterone production, to anogenital distance. (Eisenberg, *et al.*, 2011; Eisenberg, *et al.*, 2011; Mendiola, *et al.*, 2011) However, the clinical utility of such measurements remains unclear.

Azoospermia is responsible for approximately 5-20% of male infertility in the U.S.(Kolettis, 2002) While the etiologies can be diverse, they generally are categorized as obstructive or nonobstructive when treatment options are being considered. The classification usually relies on physical examination and hormone profile. However, the distinction between obstructive and nonobstructive azoospermia is uncertain and occasionally warrants a testicular biopsy to confirm a diagnosis. As measurement of anogenital distance may provide a noninvasive method to assess male reproductive

potential and testicular function, we sought to determine if AGD could be used to differentiate obstructive from nonobstructive azoospermia.

Materials and Methods

The methods of collection and cohort assembly have been reported previously.(Eisenberg, Hsieh, Walters, Krasnow & Lipshultz, 2011) Briefly, after Institutional Review Board approval was obtained from Baylor College of Medicine, eligible patients were recruited from August 2010 through November 2010 from a urology clinic specializing in reproductive medicine. Men with a history of infertility, sexual dysfunction, hypogonadism, fecundity anxiety, or vasectomy and age 18 or older were eligible. Men with a history of orchiectomy, testicular torsion, prior malignancy, prior testosterone use, or prior chemotherapy exposure were excluded. Within the cohort of men who had anogenital distance measured, we searched for men who were also azoospermic defined as an absence of sperm in the ejaculate. All men had at least two centrifuged semen analyses to confirm azoospermia. The distinction between OA and NOA was made based on history, physical examination, laboratory, and surgical findings. In all, 98 men were azoospermic. Mean age was 36.1 ± 8.0. Of the cohort 58.6% were white, 13.8% were Hispanic, and 13.8% were African American. All men provided written consent for participation.

Genital measurements

The methods of genital measurement have been previously described. Briefly, in the supine, frog-legged position with the legs abducted allowing the soles of the feet to meet, the distance from the posterior aspect of the scrotum to the anal verge was measured using a digital caliper (Neiko USA, Model No. 01407A). It is important to note that others have defined the anogenital distance (AGD) from the anus to the anterior base of the penis and the distance from the posterior scrotum to the anus (as was measured in this study) as the anoscrotal distance (ASD).(Hsieh, Breyer, Eisenberg & Baskin, 2008; Sathyanarayana, Beard, Zhou & Grady, 2010; Swan, *et al.*, 2005) The inter-rater reliability of our measurements were 0.91 for anogenital measurements at our institution. Given the age of the patients measured, the posterior scrotum was measured as the anterior border as it was felt to be a more comfortable, reliable, and reproducible measure.

From the same position, the stretched penile length (PL) was measured from the base of the dorsal surface of the penis to the tip of the glans. Testicular volume was estimated at physical examination by one investigator (LIL) in a room at approximately 25 to 27 degrees Celsius. Total testicular volume represents the sum of the right and left testes.

Hormone analysis

All hormone assays were processed by a single, experienced laboratory (Laboratory for Male Reproductive Research and Testing, Baylor College of Medicine, Houston, Texas). Testosterone (reference range: 6.9-34.7 nmol/L), LH (reference range: 6-19

mIU/mL), FSH (reference range: 4-10 mIU/mL), and estradiol (0.5-5 ng/dL) values were assessed using an automated, one-step competitive binding assay with the Beckman Coulter Access II Immunoassay system (Beckman Coulter, Inc., Brea, California). The assays were recalibrated daily with controls that spanned the normal range for all hormones.

Statistical analysis

Comparisons were made using ANOVA for most continuous variables and Chi-squared for categorical variables. Given the nonparametric distribution of the genital measures (i.e. AGD and PL), the nonparametric Mann-Whitney U-test was used for comparisons. To assess the performance of AGD length to predict the etiology of azoospermia, the data were stratified on the basis of AGD length. Multivariable logistic regression was used to determine the relationship between AGD length and etiology of azoospermia. Regression coefficients between genital measures, anthropomorphic variables, and the etiology of azoospermia were determined, and relationships with a p value < 0.2 were included in the multivariable models. All p values were two-sided. Analyses were performed using Stata 10 (StataCorp LP, College Station, Texas).

Results

In all, 98 men were azoospermic and available for analysis--69 men had obstructive azoospermia (OA) and 29 men had nonobstructive azoospermia (NOA). Mean age was 32.8 for OA men and 44.2 for NOA men. Other demographic and anthropomorphic characteristics of the OA and NOA men were similar (Table 1). Of the NOA men, 20 had karyotypes, of which 18 were normal, and 2 showed XXY. Seventeen NOA men had a Y chromosome microdeletion assessment with 3 men showing abnormalities. Of the OA men, 4 had CBAVD and 61 had previously undergone vasectomy.

Men with OA had significantly longer AGD than those with NOA (41.9 vs 36.3 mm, p=0.01). Men with OA also had significantly larger total testis size than did those with NOA (41.7 vs 26.4 mL, p<0.01). In contrast, there were no significant differences between penile length in men with OA and those with NOA (125.4 vs 123.0 mm, p=0.2, Table 2).

Frequency distribution analysis revealed that 71.1 % of men (49 of 69) with OA had an AGD • 35. In contrast, only 37.9% of men (11 of 29) with NOA had an AGD • 35. Similarly, 8.7% of men (6 of 69) with OA had an AGD < 30, while 41.4% of men (12 of 29) with NOA had an AGD < 30 (Table 3). Multivariate logistic regression analysis revealed that the odds for having obstructive azoospermia versus nonobstructive azoospermia were 5.9 (95% CI 1.01, 30.69) with an AGD < 30 mm (Table 3).

Receiver operator characteristic curve showed that AGD had an area under the curve of 0.66 (95 % CI 0.52 to 0.80). Testis size and penile size had an AUC of 0.94 (95% CI 0.89 to 0.99) and 0.58 (95 % CI 0.45 to 0.72), respectively.

Discussion

The current study demonstrated that men with nonobstructive azoospermia have a shorter AGD than do men with obstructive azoospermia. Moreover, as a single measure, an AGD cutoff of 30 mm displayed a 91% specificity for men with NOA. The relationship persisted even after adjustment for anthropomorphic and demographic variables.

During sexual development the immature genital precursors migrate ventrally via an androgen mediated pathway (Larson, 1997). The anogenital distance has been used to sex animals, because males have longer lengths than females (Greenham & Greenham, 1977; Hsieh, Breyer, Eisenberg & Baskin, 2008; Marois, 1968). Moreover, human studies in infants have established that boys have longer perineal lengths than girls (Salazar-Martinez, Romano-Riquer, Yanez-Marquez, Longnecker & Hernandez-Avila, 2004; Torres-Sanchez, Zepeda, Cebrian, Belkind-Gerson, Garcia-Hernandez, Belkind-Valdovinos & Lopez-Carrillo, 2008). Hsieh et al demonstrated shorter anogenital distances in boys with genital anomalies (i.e. hypospadias and cryptorchidism), establishing a link between normal genital development and perineal length in humans (Hsieh, Breyer, Eisenberg & Baskin, 2008). Recent data has demonstrated that AGD is also related to fatherhood, fertility and adult sperm production.(Eisenberg, Hsieh, Walters, Krasnow & Lipshultz, 2011; Mendiola, Stahlhut, Jorgensen, Liu & Swan, 2011) To our knowledge, the current report represents the first demonstration of the utility of assessing AGD in clinical practice to aid patient care.

While testicular size is an excellent predictor of the etiology of azoospermia (a fact demonstrated in the current report), men with azoospermia with a normal genital examination and a normal volume ejaculate are often offered testicular biopsy with the option for testicular sperm extraction or genital reconstruction. Other investigators have reported using testicular size coupled with FSH or testicular MRI to assist with the diagnosis of OA vs NOA.(Aaronson, *et al.*, 2010; Schoor, *et al.*, 2002) While the current report was not powered to compare available tests, it does support the value of AGD to assist in determining the etiology of azoospermia. The current data gives the urologist additional information for patient counseling regarding the etiology of azoospermia. However, it is important to note that as the AGD increases, its ability to discriminate OA from NOA diminishes. Indeed, at 35 mm, the specificity to identify NOA men is only 71% compared to 91% at 30 mm.

It is interesting to note that the men with obstructive azoospermia were taller than the men with nonobstructive azoospermia. This may have resulted by chance alone, however, it may also reflect subtle developmental differences between the groups.

Certain limitations warrant mention. Working in a referral center for male infertility, it was not always possible to blind observers to the men's diagnoses, which theoretically can have led to observer bias. Because most of the men with obstruction underwent vasectomy, the etiology of azoospermia was not a diagnostic dilemma. It is possible that men undergoing vasectomy are a population distinct from men who have obstruction for other reasons (e.g. CBAVD, idiopathic epididymal obstruction). Indeed, men with CF have been shown to have impaired testosterone production.(Boas, *et al.*,

1996) Unfortunately, the limited numbers of men remaining after stratification by obstructive diagnosis precluded subanalyses. However, future work should validate the utility of AGD in predicting azoospermia from all etiologies. In addition, there were a limited number on noncaucasian men, prevent racial subanalyses. Moreover, the current method of AGD measurement in adult men has not been studied, and thus its accuracy and reproducibility were difficult to assess by means other than comparison of measurements by investigators. (Eisenberg et al., 2011). However, we have previously shown good correlation and reproducibility of our methods. Nevertheless, our study represents the first analysis of anogenital distance in adult men and demonstrates a clinical utility for the measurement. As such, AGD may predict normal male genital development and sperm production and could therefore provide a novel metric to assess reproductive potential in men. Moreover, it may give the practitioner additional prognostic information when counseling azoospermic men.

References

Aaronson D S, Iman R, Walsh T J, Kurhanewicz J & Turek P J. (2010) A novel application of 1H magnetic resonance spectroscopy: non-invasive identification of spermatogenesis in men with non-obstructive azoospermia. *Human reproduction (Oxford, England)* **25**, 847-852.

Boas S R, Cleary D A, Lee P A & Orenstein D M. (1996) Salivary testosterone levels in male adolescents with cystic fibrosis. *Pediatrics* **97**, 361-363.

Cowin P A, Gold E, Aleksova J, O'Bryan M K, Foster P M, Scott H S & Risbridger G P. (2010) Vinclozolin exposure in utero induces postpubertal prostatitis and reduces sperm production via a reversible hormone-regulated mechanism. *Endocrinology* **151**, 783-792.

Eisenberg M L, Hsieh M H, Walters R C, Krasnow R & Lipshultz L I. (2011) The relationship between anogenital distance, fatherhood, and fertility in adult men. *PLOS One* **6**, e18973.

Eisenberg M L, Jensen T K, Walters R C, Skakkebaek N E & Lipshultz L I. (2011) The Relationship Between Anogenital Distance and Reproductive Hormone Levels in Adult Men. *The Journal of urology*.

Foster P M, Mylchreest E, Gaido K W & Sar M. (2001) Effects of phthalate esters on the developing reproductive tract of male rats. *Human reproduction update* **7**, 231-235.

Greenham L W & Greenham V. (1977) Sexing mouse pups. *Laboratory animals* **11**, 181-184.

Hsieh M H, Breyer B N, Eisenberg M L & Baskin L S. (2008) Associations among hypospadias, cryptorchidism, anogenital distance, and endocrine disruption. *Current urology reports* **9**, 137-142.

Kolettis P N. (2002) The evaluation and management of the azoospermic patient. *Journal of andrology* **23**, 293-305.

Larson W J. (1997) *Human Embryology*, p. 261-309. Churchill Livingstone, New York.

Marois G. (1968) [Action of progesterone, testosterone and estradiol on the anogenital distance and somatic sexual differentiation in rats]. *Biologie medicale* **57**, 44-90.

Martino-Andrade A J, Morais R N, Botelho G G, Muller G, Grande S W, Carpentieri G B, Leao G M & Dalsenter P R. (2009) Coadministration of active phthalates results in disruption of foetal testicular function in rats. *International journal of andrology* **32**, 704-712.

Mendiola J, Stahlhut R W, Jorgensen N, Liu F & Swan S H. (2011) Shorter Anogenital Distance Predicts Poorer Semen Quality in Young Men in Rochester, New York. *Environmental health perspectives* **119**, 958-963.

Salazar-Martinez E, Romano-Riquer P, Yanez-Marquez E, Longnecker M P & Hernandez-Avila M. (2004) Anogenital distance in human male and female newborns: a descriptive, cross-sectional study. *Environ Health* **3**, 8.

Sathyanarayana S, Beard L, Zhou C & Grady R. (2010) Measurement and correlates of ano-genital distance in healthy, newborn infants. *International journal of andrology* **33**, 317-323.

Schoor R A, Elhanbly S, Niederberger C S & Ross L S. (2002) The role of testicular biopsy in the modern management of male infertility. *The Journal of urology* **167**, 197-200.

Scott H M, Hutchison G R, Jobling M S, McKinnell C, Drake A J & Sharpe R M. (2008) Relationship between androgen action in the "male programming window," fetal sertoli cell number, and adult testis size in the rat. *Endocrinology* **149**, 5280-5287.

Swan S H, Main K M, Liu F, Stewart S L, Kruse R L, Calafat A M, Mao C S, Redmon J B, Ternand C L, Sullivan S & Teague J L. (2005) Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environmental health perspectives* **113**, 1056-1061.

Thankamony A, Ong K K, Dunger D B, Acerini C L & Hughes I A. (2009) Anogenital distance from birth to 2 years: a population study. *Environmental health perspectives* **117**, 1786-1790.

Torres-Sanchez L, Zepeda M, Cebrian M E, Belkind-Gerson J, Garcia-Hernandez R M, Belkind-Valdovinos U & Lopez-Carrillo L. (2008) Dichlorodiphenyldichloroethylene exposure during the first trimester of pregnancy alters the anal position in male infants. *Annals of the New York Academy of Sciences* **1140**, 155-162.

Tables

Table 1. Demographic, anthropomorphic, and hormonal characteristics of the cohort. Comparisons made using ANOVA for most continuous variables and Chi-squared for categorical variables with relevant p value displayed. Hormonal comparisons made using Wilcoxon rank sum test.

Table 2. Genital measurements of the cohort. Comparisons made using Wilcoxon rank sum test with relevant p value displayed.

Table 3. Test performance characteristics of AGD to distinguish etiology of azoospermia in 5 mm increments. The number (percentage) of men who have anogenital lengths below the listed cutoff are presented. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) are listed. The unadjusted and adjusted OR with 95% confidence intervals are listed showing the odds that a man will have NOA compared to OA if his AGD is below the listed cutoff. * Adjusted for age, race, and BMI. NC – not calculable

Table 1. Demographic, anthropomorphic, and hormonal characteristics of the cohort. Comparisons made using ANOVA for most continuous variables and Chi-squared for categorical variables with relevant p value displayed. Hormonal comparisons made using Wilcoxon rank sum test.

		OA		NOA		
		n	Mean (S.D.) or %	n	Mean (S.D.) or %	
Age		69	44.2 (1.1)	29	32.8 (0.9)	<0.01
Height (cm)		68	180.6 (0.8)	28	177.3 (1.3)	0.05
Weight (kg)		68	89.9 (1.5)	28	94.8 (3.6)	0.14
BMI		68	27.7 (0.5)	28	30.2 (1.2)	0.02
Race	**White**	65	94.2	20	69	0.00
	Black	3	4.4	6	20.7	
	Other	1	1.5	3	10.3	
Testosterone (nmol/L)		6	9.3 (1.0)	20	11.1 (1.2)	0.67
FSH (mIU/mL)		6	6.2 (2.2)	20	16.7 (2.5)	<0.01
LH (mIU/mL)		6	3.7 (1.3)	20	7.6 (1.2)	0.02

Table 2. Genital measurements of the cohort. Comparisons made using Wilcoxon rank sum test with relevant p value displayed.

	OA		NOA		
Genital Length	**n**	**Mean (S.D.)**	**n**	**Mean (S.D.)**	**p value**
Anogenital Distance (mm)	69	41.9 (1.4)	29	36.3 (3.0)	0.01
Stretched Penile Length (mm)	69	125.4 (2.4)	29	123.0 (4.7)	0.20
Total Testicular Volume (mL)	67	41.7 (0.8)	29	26.4 (1.3)	<0.01

Table 3. Test performance characteristics of AGD to distinguish etiology of azoospermia in 5 mm increments. The number (percentage) of men who have anogenital lengths below the listed cutoff are presented. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) are listed. The unadjusted and adjusted OR with 95% confidence intervals are listed showing the odds that a man will have NOA compared to OA if his AGD is below the listed cutoff. * Adjusted for age, race, and BMI. NC – not calculable

	OA	NOA					Unadjusted	Adjusted*
< AGD (mm)	n (%)	n (%)	Sensitivity	Specificity	PPV	NPV	OR (95% CI)	OR (95% CI)
20	0 (0)	3 (100)	10.3	100	100	72.6	NC	NC
25	1 (10)	9 (90)	31.3	98.6	90	77.3	30.6 (3.7, 256.3)	26.7 (2.1, 332.2)
30	6 (33.3)	12 (66.7)	41.4	91.3	66.7	78.8	7.4 (2.4, 22.6)	5.6 (1.0, 30.7)
35	20 (52.6)	18 (47.4)	62.1	71	47.4	81.7	4.0 (1.6, 10.0)	4.0 (0.9, 17.7)
40	34 (65.4)	18 (34.6)	62.1	50.7	34.6	76.1	1.7 (0.7, 4.1)	1.5 (0.4, 5.9)