

Total Motile Sperm Count in Adolescent Boys with Varicocele is Associated with Hormone Levels and Total Testicular Volume



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Abbreviations and Acronyms

%asymmetry = percent testicular asymmetry
AMH = anti-müllerian hormone
FSH = follicle-stimulating hormone
GnRH = gonadotropin-releasing hormone
LH = luteinizing hormone
SA = semen analysis
TMSC = total motile sperm count
TTV = total testicular volume
TVdiff = testicular volume differential

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Purpose: The risk factors for future infertility in adolescents with varicocele are controversial, and little is known about the association between hormone levels and semen parameters. Semen analysis is likely the closest marker of fertility but may be difficult to obtain in some boys secondary to personal, familial or religious reasons. Identifying other clinical surrogates for abnormal semen parameters may offer an alternative for assessing varicocele severity in these boys. We hypothesized that hormone levels and total testicular volume are predictive of abnormal total motile sperm count.

Materials and Methods: We retrospectively reviewed Tanner 5 boys with palpable left varicoceles who underwent a semen analysis and had serum hormone levels tested (luteinizing hormone, follicle-stimulating hormone, inhibin B, anti-müllerian hormone and/or total testosterone) within a 6-month period. Total testicular volume was also calculated. Abnormal total motile sperm count was defined as <9 million sperm per ejaculate.

Results: A total of 78 boys (median age 17.2 years, IQR 16.5–18.0) were included. Luteinizing hormone, anti-müllerian hormone and total testosterone were not correlated with any semen analysis parameter. There was a negative correlation between follicle-stimulating hormone and total motile sperm count ($\rho -0.35$, $p=0.004$) and positive correlation between inhibin B and total motile sperm count ($\rho 0.50$, $p <0.001$). Total testicular volume was significantly positively correlated with total motile sperm count ($\rho 0.35$, $p=0.01$). ROC analyses revealed an optimal follicle-stimulating hormone cutoff of 2.9, an optimal inhibin B cutoff of 204 and an optimal total testicular volume cutoff of 34.4 cc to predict abnormal total motile sperm count.

Conclusions: Total motile sperm count is inversely associated with follicle-stimulating hormone levels, and directly associated with inhibin B levels and total testicular volume. Optimized cutoffs for serum follicle-stimulating hormone, inhibin B and total testicular volume may prove to be reasonable

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surrogates for total motile sperm count in boys who defer semen analysis for personal or religious/cultural reasons.

Key Words: varicocele, semen analysis, hormones, follicle stimulating hormone, sperm count

VARICOCELES affect 15% of men in the general population and an even higher percentage of infertile men. In pediatric urology practice, varicoceles are found in 14% to 29% of adolescent boys at the end of puberty.^{1–3} The risk factors for future infertility in adolescents with varicocele and the indications for treatment of varicoceles during adolescence are controversial. Over the past several decades, various clinical markers have been proposed as indications for varicocele severity or treatment. Semen analysis is likely the closest marker to fertility available in adolescent boys in the absence of attempting paternity but even abnormal semen parameters do not necessarily indicate future fertility issues. Furthermore, semen analysis may be difficult to obtain in some boys secondary to personal, familial or religious reasons. Additionally, physician comfort with asking for a semen analysis and parent/patient knowledge about this test may present additional barriers to obtaining a semen analysis.⁴ Identifying other clinical surrogates for abnormal semen parameters may offer an alternative for assessing varicocele severity in boys unable to provide semen samples. In 2007 Diamond et al found significant correlations between percent testicular asymmetry and semen quality.⁵ Additionally, total testicular volume and testicular volume differential have been found in other studies to be associated with total motile sperm count.^{6,7}

Despite these reports, the association between hormone levels and semen parameters in adolescent boys remains largely unknown. Guarino et al, in one of the only studies of adolescent boys with varicoceles, noted a significantly increased stimulated FSH in response to GnRH as well as basal levels of LH and FSH in boys with varicoceles and abnormal semen parameters compared to boys with varicoceles and normal semen parameters.⁸ Other studies of adults with varicoceles have analyzed hormone levels (testosterone, FSH, LH and prolactin) in combination with other factors to determine risk factors for abnormal SA parameters or as markers for improvement in semen quality after varicocelectomy.^{9–12} We investigated if any such relationship exists in a cohort of postpubertal boys with varicoceles. We hypothesized that hormone levels (specifically FSH, LH and inhibin B), total testicular volume and testicular volume differential would be associated with abnormal TMSC.

MATERIALS AND METHODS

We retrospectively reviewed our institutional review board approved database of varicocele cases seen at a single

institution from January 2014 to June 2017. All Tanner 5 boys (15 to 19 years old) with palpable left varicoceles who underwent a SA and had serum hormone levels obtained within a 6-month period of the SA were included. We excluded any boy with a subclinical left varicocele or bilateral varicoceles. If more than 1 SA result was available within the 6-month window, the SA with the best results was used. Starting prior to 2014, the Division of Pediatric Urology at the Children's Hospital of Philadelphia followed a departmental protocol for evaluation and followup of all patients with varicoceles. As part of this protocol, all boys with palpable left varicoceles who are at least 15 years old and Tanner 5 are recommended to obtain serum hormone studies including LH, FSH, inhibin B, AMH and total testosterone at the time of semen analysis. In patients with scrotal ultrasound or orchidometer measurements within 6 months of the semen analysis the TTV, TVdiff and % asymmetry were calculated. For %asymmetry the following equation was used: (right testis volume – left testis volume)/total testicular volume. When both measurements were obtained, ultrasound measurements were used for analysis.

Serum hormone levels, TTV, TVdiff and %asymmetry were correlated with semen parameters, including total volume, percent motility and total motile sperm count using Spearman rank correlation. The Mann-Whitney U test was used to compare continuous variables between groups. ROC analysis was used to analyze the ability of hormone levels and/or testicular volume measurements to predict abnormal TMSC (defined as <9 million sperm/ejaculate based on WHO 2010 criteria for minimal reference ranges for total volume [1.5 ml], sperm concentration [15 million/ml] and percent motility [40%]).^{13,14} To determine optimal cutoffs for ROC curves, the Youden index was calculated. The Youden J statistic is a method that maximizes the sum of the sensitivity and specificity when determining the optimal cutoff of a diagnostic test.^{15–17} All statistics were performed with Stata® 14.2.

RESULTS

In total 117 Tanner 5 boys with palpable left varicoceles met eligibility criteria during the study period. Of this group 25 did not obtain a SA. A total of 92 boys underwent SA, and of this group 78 also underwent serum hormone studies within 6 months of the SA. Baseline characteristics for all 78 boys are shown in table 1. Median patient age was 17.2 years (IQR 16.5–17.9) and median TMSC was 14.7 (3.7–36.2). In total, 29 of the 78 boys (37%) had TMSC less than 9 million per ejaculate and met criteria for abnormal semen analysis. LH and AMH were not significantly correlated with any SA parameter. FSH was weakly negatively correlated with both total sperm count (ρ –0.38, $p=0.005$) and

Table 1. Baseline cohort characteristics

No. pts	78	
Median yrs age at SA (IQR)	17.2 (16.5–17.9)	
No. varicocele grade (%):		
I	8 (10)	
II	27 (34)	
III	41 (53)	
Unknown	2 (3)	
Median cc testis vol (IQR) (71 pts):		
Rt	20.0 (15.0–25.0)	
Lt	17.2 (13.0–22.0)	
TTV	38.3 (29.6–49.7)	
No. method of obtaining testis vol (%) (71 pts):		
Scrotal ultrasound	59 (83)	
Orchidometer	12 (17)	
Median semen parameters (IQR):		2010 WHO 5th percentile
Vol (ml)	1.8 (1–2.7)	1.5 ml
Concentration (million/ml)	20 (10–38)	15 Million/ml
% Total motility	51 (41–60)	40%
TMSC (million/ejaculate):	14.7 (3.7–36.2)	9 Million/ejaculate
No. TMSC <9 million/ejaculate (%)	29 (37)	
Median serum hormone levels (IQR):		Laboratory range (male, Tanner 5)
LH (mIU/ml)	3.8 (2.7–4.9)	1.7–8.6 mIU/ml
FSH (mIU/ml)	3.6 (2.2–5)	1.5–12.4 mIU/ml
Inhibin B (pg/ml)	188 (142–234)	66.9–300 pg/ml
AMH (ng/ml)	8.1 (4.8–10.6)	0.11–13.07 ng/ml
Total testosterone (ng/dl)	524 (386–685)	188–882 ng/dl

TMSC ($\rho -0.35$, $p=0.004$). Total testosterone level was only negatively correlated with percent sperm motility, although the relationship was weak ($\rho -0.29$, $p=0.01$). The strongest positive correlations were noted between inhibin B levels and SA parameters. Inhibin B was moderately correlated with total sperm count ($\rho 0.52$, $p <0.001$) and TMSC ($\rho 0.50$, $p <0.001$).

In the 71 boys who also had testicular volume measurements TTV was significantly positively correlated with TMSC ($\rho 0.35$, $p=0.01$). No other testicular volume measurements including TVdiff and %asymmetry were correlated with any SA parameters. In addition, no testicular volume parameter was significantly correlated with any serum hormone studies.

When comparing boys with TMSC <9 to boys with TMSC ≥ 9 , serum FSH and inhibin B levels were the only hormone levels that were significantly different between the groups (table 2). Median TTV was also significantly different between TMSC <9 boys and TMSC ≥ 9 boys (30.6 cc vs 41.0 cc, $p <0.01$).

All other serum levels, including LH, AMH and total testosterone, as well as varicocele grade were similar between the 2 groups.

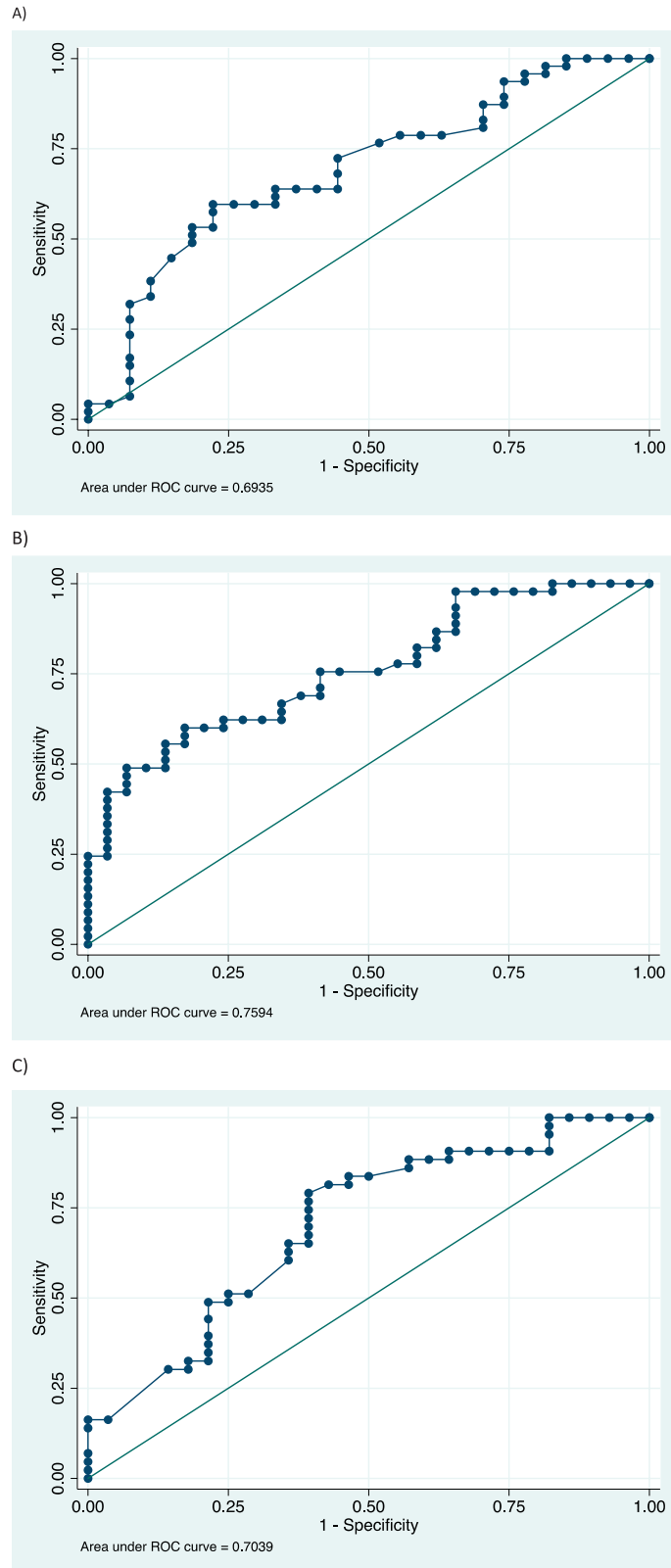
Given these findings, we next generated ROC curves to determine optimal cutoffs for FSH, inhibin B and TTV separately in predicting abnormal TMSC. TMSC <9 million sperm/ejaculate was used as a cutoff for abnormal TMSC as described in the Methods section. ROC analysis revealed an optimal FSH cutoff of 2.9 to predict abnormal TMSC (AUC 0.69, 95% CI 0.57–0.82). With this cutoff a FSH >2.9 had 60% specificity and 78% sensitivity in predicting an abnormal TMSC (part A of figure). Similar analyses revealed an optimal cutoff of 204 for inhibin B (AUC 0.76, 95% CI 0.65–0.87, sensitivity 60% and specificity 83%, part B of figure) and a cutoff of 34.4 cc for TTV (AUC 0.70, 95% CI 0.58–0.83, sensitivity 79% and specificity 61%, part C of figure).

Table 3 shows the optimized cutoff values for each risk factor, as well as the percentage of patients in the cohort with abnormal or normal TMSC based on

Table 2. Median TTV and hormone levels based on TMSC group

	TMSC <9 Million (Abnormal)	TMSC ≥ 9 Million (Normal)	p Value
Median yrs age at SA (IQR)	17.4 (16.7–18.2)	17.2 (16.3–17.9)	0.09
Median cc TTV (IQR)	30.6 (25.6–41.3)	41.0 (35.1–50.0)	<0.01
% Grade III varicocele	48.3	55.6	0.79
Median serum hormone levels (IQR):			
LH (mIU/ml)	3.7 (2.8–4.6)	3.8 (2.7–5.3)	0.59
FSH (mIU/ml)	4.4 (3–6.5)	2.6 (1.9–4.4)	<0.01
Inhibin B (pg/ml)	163 (103–196)	211 (158–264)	<0.01
AMH (ng/ml)	8.7 (6.1–13.5)	8.1 (4.7–10.2)	0.41
Total testosterone (ng/dl)	514 (370–719)	523 (407–683)	0.99

p Values were calculated using Mann-Whitney U test for continuous variables and Fisher exact test for categorical variables (ie varicocele grade).



ROC curves. *A*, ROC curve of FSH as predictor of abnormal TMSC (defined as <9 million sperm per ejaculate). AUC was 0.69 (95% CI 0.57–0.82) and Youden index analysis determined ideal cutoff of 2.9 as predictor of abnormal TMSC. *B*, ROC curve of inhibin B as predictor of abnormal TMSC (defined as <9 million sperm per ejaculate). AUC was 0.76 (95% CI 0.65–0.87) and Youden index analysis determined ideal cutoff of 204 as predictor of abnormal TMSC. *C*, ROC curve of TTV as predictor of abnormal TMSC (defined as <9 million sperm per ejaculate). AUC was 0.70 (95% CI 0.58–0.83) and Youden index analysis determined ideal cutoff of 34.4 as predictor of abnormal TMSC.

Table 3. Percentage of patients with normal vs abnormal TMSC (defined as <9 million sperm/ejaculate) based on presence of risk factors

Factors Present at SA	No. Pts with Normal TMSC (%)	No. Pts with Abnormal TMSC (%)
None	18 (100)	0 (0)
Only 1	13 (68)	6 (32)
2	7 (33)	14 (67)
All 3	4 (31)	9 (69)

Risk factors consisted of FSH >2.9 mIU/ml, inhibin B <204 pg/ml and TTV <34.4 cc.

the presence of none, 1, 2 or 3 of the factors at time of obtaining SA. For patients with none of the risk factors all 18 (100%) had normal TMSC, while for patients with all 3 of the risk factors only 31% had normal TMSC.

DISCUSSION

The ultimate goal of varicocele treatment is preserving or restoring fertility. In adolescents with varicocele there is controversy over what clinical parameters or findings will be associated with future infertility. The American Urological Association/American Society for Reproductive Medicine “Best Practice Policy” from 2001 and the more recent “Report on Varicocele and Infertility” from the American Society for Reproductive Medicine and the Society for Male Reproduction and Urology provide 2 indications for varicocele treatment in adolescent boys—objective evidence of reduced ipsilateral testicular size or semen abnormality.^{18,19} Neither indication provides a cutoff value or definition of what is meant by “reduced size” or semen “abnormality.”

Semen analysis is likely the closest marker to fertility available in adolescent boys in the absence of attempting paternity but even abnormal semen parameters do not necessarily indicate future fertility issues. Based on the WHO guideline, reference ranges for semen parameters can be determined but the ideal value in adolescents is unclear.¹³ Importantly, Chu et al recently showed that semen parameters can normalize over time in up to 47% of Tanner 5 boys with initial abnormal parameters, without surgical repair.²⁰

Despite their importance in evaluating post-pubertal boys with varicoceles, SAs are not always obtained. Difficulty in obtaining SA may be secondary to personal, familial or religious reasons, and even secondary to physician barriers.⁴ Furthermore, the process of SA acquisition involves time, special facilities and possible costs to the patient/family. For these reasons surrogate markers for abnormal semen parameters may obviate the need for SA (or repeat analyses) and provide an alternative to patients unable or unwilling to provide samples.

Testicular volumes can be obtained with an orchidometer during physical examination or ultrasonography, although some studies have shown variability with each technique and likely higher accuracy with ultrasound measurements.^{21–23} In 2007 Diamond et al found significant correlations between %asymmetry and semen quality.⁵ Boys with greater than 10% asymmetry had significantly lower sperm concentration and TMSC than boys with less than 10% asymmetry, and these decreases were worse for boys with >20% asymmetry.⁵ Additionally, TTV and TVdiff have been found in other studies to be associated with TMSC.^{6,7}

Hormone dysfunction in and of itself has been long considered as a potential marker for varicocele severity and has been proposed as an indicator for surgical correction by some. Kass et al were the first to perform GnRH stimulation tests in boys with varicoceles, and they reported exaggerated LH and FSH responses after stimulation in 31% of boys 10 to 19 years old with palpable varicoceles.²⁴ However, baseline LH, FSH and testosterone levels have not been shown to be consistently different between boys with varicoceles and age or Tanner matched controls.^{25,26} Studies of infertile men with varicoceles and hypogonadism show significant improvements in serum testosterone after surgical repair.²⁷ Interestingly, Guarino et al reported significantly increased stimulated FSH in response to GnRH as well as basal levels of LH and FSH in boys with varicoceles and abnormal semen parameters compared to boys with varicoceles and normal semen parameters.⁸

In addition to FSH, LH, testosterone and the GnRH stimulation test, serum inhibin B levels have been suggested as an alternative to the use of basal and stimulated gonadotropin levels. Inhibin B is secreted by Sertoli cells and is involved in negative feedback control of FSH production by the pituitary. One study found no differences in basal or GnRH stimulated FSH and LH, or basal testosterone between pubertal boys (Tanner 4 or 5) with varicoceles and age matched controls, although it did find a significant reduction in inhibin B levels in the boys with varicoceles compared to controls.²⁶ This study also showed a significant correlation between inhibin B levels and testes volume, suggesting that inhibin B level may be an early marker for Sertoli cell damage.

Several studies have analyzed hormone dysfunction in men with varicoceles and correlated these findings to semen parameters.^{9–12} Damsgaard et al performed a cross-sectional, multi-institutional, multinational study of 7,035 European men (all >18 years old) and noted that worsening severity of varicocele grade was associated with higher serum FSH levels, lower serum inhibin B levels and higher serum LH levels.¹⁰ Interestingly, the authors did not find any significant

difference between serum testosterone and free testosterone between men with vs without varicocele. In another study in adults (mean age 35 years) undergoing varicocelectomy for infertility, serum inhibin B levels increased after varicocelectomy and were associated with improvement in semen parameters.¹¹ While these studies in adults are compelling, the generalization of their findings to adolescent patients with varicocele not referred for fertility concerns is unclear, and the lack of studies in adolescent boys with varicoceles comparing hormone levels and semen parameters was a primary driving force behind the present study.

In this study we found several significant correlations between hormone levels and semen parameters. Although most correlations were weak (FSH, TTV), inhibin B had a moderate positive correlation with TMSC. Total testosterone was only found to have a weak negative correlation with percent sperm motility. Given that TMSC is used most commonly in the literature and is thought by many to represent the most important semen parameter, we focused on this parameter in our ROC analyses and for designing a risk model. Using the WHO 2010 criteria and recent publications as guidance, an abnormal TMSC was defined as <9 million sperm per ejaculate.^{13,14} With this definition, ROC curves were generated for FSH, inhibin B and TTV.

In our analyses the optimal cutoffs were FSH >2.9 mIU/ml, inhibin B <204 pg/ml and TTV <34.4 cc. Interestingly, when we used these cutoffs to evaluate our patients, all 18 boys (100%) who had none of these abnormal factors had normal TMSC, while for boys with all 3 abnormal factors 31% had normal TMSC and 69% had abnormal TMSC.

We believe that these cutoffs for FSH, inhibin B and TTV in assessing risk for abnormal TMSC may be helpful in situations in which semen analyses cannot be obtained in Tanner 5 boys with varicoceles, with the understanding that none of the factors is able to determine all normal or abnormal TMSC patients. Furthermore, we are not proposing the

elimination of semen analysis in the evaluation of the adolescent varicocele, but rather we are suggesting that serum levels of FSH and inhibin B and TTV may serve as possible markers for abnormal TMSC, and may play a role in the followup of patients with abnormal semen parameters. Understanding that there are still limitations to these markers and cutoffs, if there are no cultural, religious or personal reasons not to obtain a SA, we continue to recommend obtaining a SA in all Tanner 5 boys who are over 15 years of age with palpable left varicoceles.

This study has several limitations, including its retrospective nature and small sample size. Our results are only generalizable to Tanner 5 boys who are over 15 years of age with palpable varicoceles, and should not be used for boys at other stages of puberty given the variability in hormone and semen parameters that can occur during pubertal development. Furthermore, hormone levels at time of semen analysis were included but we did not analyze repeat hormone levels. Another limitation of our study is that we combined orchidometer and ultrasound measurements for testicular volume. It has been our practice to minimize use of ultrasound and not to obtain repeat ultrasounds annually. Therefore, combining these measurements is a truer reflection of our clinical practice but may introduce measurement bias. Lastly, use of a single semen analysis may lead to incorrect conclusions about semen parameters, and while it is our practice to repeat all abnormal semen analyses, this was not done in all cases and may limit the generalizability of the results.

CONCLUSIONS

TMSC, which is thought to be the most important semen parameter related to fertility, is inversely correlated with serum FSH levels and directly correlated with inhibin B levels and TTV. While semen analysis remains the closest surrogate of future fertility in adolescent boys with varicoceles, serum FSH, serum inhibin B and TTV may prove to be reasonable surrogates for TMSC in boys who defer SA for personal or religious/cultural reasons.

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