Preoperative follicle-stimulating hormone: A factor associated with semen parameter improvement after microscopic subinguinal varicocelectomy

Vinayak Madhusoodanan[1]; Ruben Blachman-Braun[1]; Premal Patel[2]; Lunan Ji[2]; Thomas A. Masterson[2]; Michael Owyong[1]; Aubrey Greer[1]; Ranjith Ramasamy[2]
[1]University of Miami Miller School of Medicine, Miami, FL, United States; [2]Department of Urology, University of Miami Miller School of Medicine, Miami, FL, United States

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Abstract

Introduction: Currently, there exists no serum biomarker to predict patients likely to benefit from varicocelectomy. The purpose of this study was to assess the association between baseline follicle-stimulating hormone (FSH) and semen parameter changes after subinguinal microscopic varicocelectomy.
Methods: We retrospectively reviewed all men who underwent microscopic subinguinal varicocelectomy between August 2015 and October 2018. Pre- and postoperative semen analyses were stratified per total motile sperm count (TMSC): TMSC <5, 5–9, and >9 million (based on TMSC required for in vitro fertilization, intrauterine insemination (IUI), and natural conception, respectively). Then, variables were analyzed to determine the correlation with postoperative TMSC values and upgrade in TMSC category.
Results: Among the 66 men analyzed, 55 (83.3%) and 11 (16.7%) had a preoperative TMSC of <5 million and 5–9 million, respectively. A total of 33 (50%) patients upgraded in TMSC category, 26 of them achieving levels corresponding to natural conception and seven achieving those of IUI. Additionally, a significant correlation was observed between postoperative TMSC and preoperative TMSC (r=0.528; p<0.001), and preoperative FSH (r=-0.314; p=0.010). A lower preoperative FSH (odds ratio [OR] 0.82; 95% confidence interval [CI] 0.68–0.98; p=0.028) and a higher preoperative TMSC (OR 1.37; 95% CI 1.06–1.76; p=0.015) were associated with upgrade in TMSC category.
Conclusions: Lower preoperative FSH and higher TMSC are associated with improvement in TMSC category after varicocelectomy, although small sample size limited the study. FSH can be useful to identify men who are most likely to benefit from varicocele repair.

Introduction

The prevalence of varicoceles in men is approximately 15%.[1,2] Although, the presence of a varicocele does not necessarily lead to male infertility, the prevalence of varicoceles among men with infertility is significantly higher, with up to 44% in those with primary infertility and up to 81% in those with secondary infertility.[3,4] The incidence of varicoceles also appears to increase with age, with a higher incidence in the elderly population.[5] In 1955, Tulloch was the first to describe the treatment of male infertility using varicocelectomy.[6] Since then, several techniques for varicocele repair have been described, including through inguinal, subinguinal, laparoscopic and microsurgical approaches, as well as through embolization. In infertile men with abnormal semen parameters and palpable varicoceles, varicocelectomies have been shown to significantly improve both semen analysis parameters and spontaneous pregnancy rates.[7,8] Varicocelectomies have also been shown to have similar, clinically significant outcomes in subclinical varicoceles.[9] That said, not all infertile men who undergo varicocele repair show improvements in semen analysis parameters, and spontaneous fertility rates remain low.[10] Currently, excluding varicocele grade, no pre-operative parameter has been identified as a reliable predictor of patients who are more likely to benefit from varicocele repair.[11]

Follicle-stimulating hormone (FSH) is a gonadotropin hormone produced by the anterior pituitary gland. It plays an important role in stimulating spermatogenesis and is involved in the negative feedback loop of the hypothalamic pituitary gonadal axis.[12] As such, FSH levels can serve as a surrogate marker of the degree of spermatogenesis, and elevated levels could indicate impaired spermatogenesis. The normal reference range for FSH levels in males varies considerably between laboratories, with the upper end of normal ranging from 8 IU/L to 18 IU/L. Even within normal reference ranges, higher FSH levels correlate with increased risk for abnormal semen analysis.[13] In a healthy population, men with grade II and III varicoceles have been found to have higher median FSH levels compared to those with absent or only Grade I varicoceles.[2]

In this study, we aim to analyze different demographic and clinical variables (i.e. preoperative semen analysis, FSH, LH, testosterone, varicocele grading, testes volume) and their correlation with post-operative semen parameters and association with upgrade in total motile sperm count (TMSC) category following microscopic subinguinal varicocelectomy.

Methods

After obtaining IRB approval, a retrospective chart review was performed including all men who underwent microsurgical subinguinal varicocelectomy between August 2015 and October 2018. All procedures were performed by a single surgeon, and all varicoceles for repair were clinically palpable or detected by ultrasound, only if physical exam demonstrated a tight scrotum. Men with pre-operative TMSC >9 million and/or subclinical varicoceles were excluded from the study.

Before surgery, all patients underwent an evaluation that consisted of a physical exam, hormonal profile and semen analysis. Physical exam was used to determine testicular volume, using an orchidometer, in cubic centimeters (cc), laterality, and varicocele grade. For patients with bilateral varicoceles, the highest grade was used to determine that patient's overall varicocele grade. The pre-operative hormone profile including FSH, luteinizing hormone (LH) and testosterone.

The semen analysis parameters (at least 2 prior to surgery) that were recorded included volume, TMSC, concentration, and percent motility—the mean of the results of both studies was utilized for the data analysis. On follow-up after surgery, semen analyses were obtained within 3-6 months. Patients were instructed to remain abstinent for at least 2 days prior to providing pre-operative and post-operative semen samples. A single lab technician performed both semen analyses for all patients to minimize inter-observer variability.

Based on TMSC results from semen analysis, patients were categorized based upon corresponding candidacy for assisted reproductive technology (ART): in vitro fertilization (IVF, TMSC <5 million), intrauterine insemination (IUI, 5-9 million) and natural conception (>9 million). The primary outcome of the study was an upgrade in TMSC categories from pre-operative to post-operative semen analyses. This method of categorizing and then classifying patients in regards to achievement of upgrade was based on prior studies by Samplaski et al. and Masterson et al., in which the utility of varicocelectomy in allowing couples to use less invasive forms of ART was shown to be a primary benefit.[14,15]

Statistical analysis

Statistical analysis was performed with the SPSS version 24.0 (Chicago, USA). Means and standard deviations (± SD) or medians and interquartile ranges [25-75] were calculated per the data distribution. Comparisons of the numerical variables between the group of patients that upgraded in TMSC category against the group that did not upgrade in TMSC category were performed using the Mann-Whitney U or Student T test as required. Categorical variables were presented as absolute values and frequencies, and were analyzed with a Chi-square test. Furthermore, a Spearman correlational analysis was performed within the continuous variables and post-operative TMSC. Then, a multivariable-adjusted logistical regression analysis was performed to calculate the risk [Odds ratio (OR)] for having a TMSC category upgraded. After that, to calculate the positive likelihood ratio (+LR) for TMSC category upgrade, patients were stratified based on achievement of upgrade into a 2 x 2 table based on the different FSH pre-operative cut-off values [FSH ≤ the cut-off value that upgraded as A, FSH ≤ the cut-off value that did not upgrade as B, FSH > the cut-off value that upgraded as C, and FSH > the cut-off value that did not upgrade as D], and the +LR for upgrading of TMSC category for the different pre-operative FSH ≤ the cut-off values were calculated .[16] For this research, a p-value < 0.05 was considered statistically significant.

Results

A total of 66 men who underwent microscopic subinguinal varicocelectomy were analyzed. Of the 55 patients initially classified within the IVF category, 18 (32.7%) achieved a TMSC corresponding to natural pregnancy, and 7 (12.7%) achieved that of IUI at 3-6 months after surgery. Of the 11 patients initially qualifying for the IUI category, 8 achieved a TMSC corresponding to natural conception (Table 1). Thus, 33 (50%) patients were considered in the TMSC upgraded group.

Overall, the mean age at surgery was 35.5 ± 7.3 years, median testicular volume was 14 [12 - 16] cc, bilateral varicoceles were present in 19 (28.8%) patients, and the distribution of the varicoceles per grade were I = 20 (30.3%), II = 26 (39.4%) and III = 20 (30.3%). When comparing both groups there was no statistically significant difference between age at surgery (p = 0.264), testicular volume (p = 0.642), varicocele laterality (p = 0.415), or grading (p = 0.449). Even though an overall lower pre-operative FSH was observed in the TMSC upgraded group, this was not statistically significant (upgraded: 6.5 [4 - 8.7] (range: 1.8 – 15.9) vs. not upgraded: 7.4 [4.9 - 14.2] (range: 1.9 – 27.7); p = 0.119). Although in the pre-operative semen there was no difference in the volume between groups, sperm concentration (4.5 vs. 1; p = 0.001), total motility (20 vs. 6.4; p = 0.009) and TMSC (2.2 vs. 0.2; p = 0.002) were found to be significantly better in the patients who achieved an improvement of TMSC category (Table 2).

Then, after performing a correlational analysis between post-operative TMSC and other continuous variables, it was shown that age at surgery (r = -0.164 ; p = 0.189), testes volume (r = 0.162; p = 193), semen volume (r = -0.087; p = 0.485), pre-operative testosterone (r = 0.095; p = 0.452), and pre-operative LH (r = -0.116; p = 0.400) were not statistically correlated with post-operative TMSC. On the other hand, a statistically significant correlation was observed between post-operative TMSC and pre-operative TMSC (r = 0.528; p < 0.001), sperm concentration (r = 0.528; p < 0.001), total motility (r = 0.404; p = 0.001), and pre-operative FSH (r = -0.314; p = 0.010). After performing a multivariable adjusted analysis, we found pre-operative TMSC and FSH to be associated with post-operative TMSC category improvement.

Our analysis indicates that higher pre-operative TMSC is associated with better post-operative TMSC outcomes; for each million unit increase in pre-operative TMSC there is a risk of OR = 1.37 (95% CI: 1.06 - 1.76; p = 0.015) improvement of TMSC category, favoring improvement. On the other hand, higher pre-operative FSH is associated with worse outcomes, suggesting that for each IU/ml increase of pre-operative FSH, there is a risk, OR = 0.82 (95% CI: 0.68 - 0.98; p = 0.028) of TMSC category improvement (Table 3). Furthermore, the tendency of a lower pre-operative FSH to associate with better outcomes persists when analyzing the patients that had an improvement of TMSC category. The +LR of upgrading TMSC category decreases as the pre-operative concentration of FSH increases, with the highest +LR of upgrading TMSC category associated with a FSH ≤ 11 IU/ml (+LR = 1.40), while patients with FSH ≤ 16 IU/ml had a +LR under the unit (+LR = 1.18) (Table 4).

Discussion

According to guidelines from the American Society for Reproductive Medicine (ASRM), varicocelectomy is indicated in men with infertility or desire for future fertility, with a palpable varicocele, whose timeline to conception is not a concern, and who has abnormal semen parameters.[10] Varicocelectomy has been demonstrated as an effective treatment for infertile men; one meta-analysis shows the natural pregnancy rate remains low at 33% in treated patients compared to 15.5% in the control group.[8] Increasing varicocele grade has been the most studied male prognostic factor of response to varicocelectomy,[17] however, new evidence suggests that other factors, including baseline sperm density and reproductive hormones, may have a prognostic utility for varicocelectomy response.[18] Furthermore, infertile men with varicoceles may also have concomitant causes of infertility and varicocele repair alone may be insufficient to achieve fertility in these men. In a study of 1,213 men with infertility, 35.6% had idiopathic infertility with no clearly identifiable cause.[19] Alongside semen parameter decreases in volume, motility, count and normal morphology, FSH level has also been shown to increase as a man ages.[20,21]

In our study, we stratified men who underwent microsurgical varicocelectomy into their respective pre-operative TMSC category (< 5 million, 5-9 million, and > 9 million) and assessed predictors of TMSC improvement. We found pre-operative TMSC and FSH was scientifically correlated with postoperative TMSC, as higher pre-operative TMSC and lower FSH are associated with an improvement in TMSC category post-operatively. Our findings are in line with the other reports which suggest that higher baseline sperm parameters are associated with better post-operative semen analysis.[18] Furthermore, in patients with varicoceles, pre-operative FSH levels may serve as an indirect marker for spermatogenesis with elevated levels suggesting a higher degree of dysfunction with a lower likelihood of improvement after varicocelectomy. In a study performed in 123 patients by Shabana et al., FSH in those that responded to varicocelectomy was slightly lower than non-responders (7.4 IU/L vs. 7.9 IU/L; $p = 0.0833$), though their univariable analysis, similar to that of this study, did not reach statistical significance.[17] Our results scientifically indicate that pre-operative FSH can be a predictive factor of post-operative TMSC, as a lower FSH ($r = -0.314$; $p = 0.010$) scientifically correlated with post-operative TMSC ($r = -0.314$; $p = 0.010$) and associated with TMSC category upgrade. Though, in our study varicocele grade was non-significant, this might be attributed to the variability in physical exam and the absence of an objective measurement of varicocele grading. Markers of spermatogenesis as predictors of response to varicocelectomy have not been extensively studied. Okada et al. evaluated pre-operative inhibin B and found that it was not a reliable predictor of response to varicocelectomy, however this was done in a small sample of patients and follow-up is needed. That study also found that responders to varicocelectomy had significantly lower pre-operative FSH levels compared to non-responders (5.8 IU/L vs 8.9 IU/L; $p < 0.05$), although post-operative FSH did not change significantly from pre-operative levels in

either responders and non-responders.[22]

The exact mechanism of how varicoceles affect spermatogenesis is not completely understood, but is likely multifactorial.[23] Some authors have hypothesized that varicocele's effect on spermatogenesis has a temporal and progressive effect, pointing to the significantly higher prevalence of varicoceles among men presenting with secondary infertility compared to primary infertility.[4,24] Men with varicoceles have been shown to have increased testicular temperature and testicular hypoperfusion, both of which could have a negative impact on spermatogenesis through an effect on DNA repair potential and local ischemia, respectively.[23] Increased oxidative stress also appears to play a role in the pathogenesis of varicoceles.[25] The presence of anti-sperm antibodies has also been found more frequently in infertile men with varicoceles, and varicocele repair may lead to a reduction in the level of these antibodies.[26,27] Our data indicates that within patients with varicoceles who have abnormal semen parameters, pre-operative FSH levels are potentially useful in identifying those who are most likely to benefit from microsurgical varicocelectomy. In our cohort, the results suggest that for each IU/mL increase in FSH, there was a decreased chance of upgrading TMSC category, OR = 0.82 ($p = 0.026$). Although we observed a tendency that indicates that lower values of pre-operative FSH are associated with improvement of TMSC category, within the patients that improved TMSC categories most had lower values of FSH, and patients with an FSH $\leq$ 11 IU/ml had a higher chance of upgrading of TMSC category (+LR = 1.40). Furthermore, in our cohort all the patients that upgraded in TMSC category had a pre-operative FSH $\leq$ 16 IU/ml (+LR = 1.18).

Nonetheless, the change in the +LR observed as the FSH cut-off value increases should be interpreted in the context of the relatively sample size of this study and in context of the other clinical variables of patient presentation. Thus, men with lower pre-operative FSH might have better post-operative outcomes, although patients with higher pre-operative FSH levels might still benefit from microsurgical varicocelectomy, although they may not have as favorable a likelihood of upgrading in TMSC category as those with lower pre-operative FSH values. Thus, pre-operative FSH could serve as part of the predictor tools that help counsel infertile men who are considering varicocele repair, and identify those patients that have a higher likelihood of improvement of TMSC category.

Despite the strengths of the study which include that all the physical evaluations and procedures were performed by a single surgeon, and that all semen analysis were performed by the same lab technician, limitations of the study need to be stated. Limitations included the inherent boundaries of a retrospective study, data from a single center, limited sample size, and variability in physical exam. Although our results seem reliable (relatively narrow confidence intervals given the sample size), further studies should evaluate the relationship in a sample of more than 300 patients to provide reliable results. Of course, improvement in semen parameters may not translate into improved pregnancy rate; for this reason we hope that further multicenter randomized control trials, performed in larger cohorts, analyze the association of post-operative

TMSC and pregnancy rate with different pre-operative variables such as semen parameters, serum hormones concentrations (i.e., FSH, LH, testosterone, inhibin B), testicular volume, and varicocele grading measured with ultrasound.

Conclusions

Our study suggested associations between both lower pre-operative FSH and higher pre-operative TMSC in men who upgraded in ART-based TMSC categories after microsurgical varicocelectomy. Lower FSH levels can suggest preserved spermatogenesis and may be a useful pre-operative prognostic tool to help identify those men with abnormal semen parameters who are most likely to benefit from varicocele repair.

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Figures and Tables

Table 1. Changes in assisted reproductive technology candidacy after varicocele repair				
	Category after varicocelectomy			
Preoperative category	**Patients, n**	**IVF (<5 million)**	**IUI (5–9 million)**	**Natural pregnancy (> 9 million)**
IVF (<5 million)	55	30 (54.5%)	7 (12.7%)	18 (32.7%)
IUI (5–9 million)	11	1 (9.1%)	2 (18.2%)	8 (72.7%)

IUI: intrauterine insemination; IVF: in vitro fertilization.

Table 2. A comparison of clinical, demographic, and semen analysis results between the patients who did not upgrade TMSC categories from preoperative to postoperative periods vs. those who upgraded TMSC category			
	TMSC not upgraded n=33 (%)	**TMSC upgraded** n=33 (%)	**p**
Age in years	36.5±7.9	34.5±6.7	0.264
Highest varicocele grade			
I	12 (36.4)	8 (24.2)	
II	13 (39.4)	13 (39.4)	
III	8 (24.2)	12 (36.4)	0.449
Laterality			
Unilateral	22 (66.7)	25 (75.8)	
Bilateral	11 (33.3)	8 (24.2)	0.415
Testes volume (cc)	14 (12–15)	14 (12–16)	0.642
Testosterone (ng/dl)	421 (300.5–605)	439.5 (355.5–547.3)	0.427
FSH (IU/ml)	7.4 (4.9–14.2)	6.5 (4–8.7)	0.119
LH (IU/ml)	4.9 (3.4–7.5)	4.5 (3.4–6.1)	0.980
Pre-operative semen analysis characteristics			
Volume (cc)	2.8 (2–3.5)	2.3 (1.5–3.5)	0.278
Concentration (million/c)	1 (0.3–4.8)	4.5 (2–11.7)	**0.001**
Total motility	6.4 (1.8–16.5)	20 (5.5–38)	**0.009**
TMSC (million)	0.2 (0.01–1.8)	2.2 (0.5–5.4)	**0.002**

FSH: follicle-stimulating hormone; LH: luteinizing hormone; TMSC: total motile sperm count. Mean and ± standard deviation, median and interquartile range [25-75].

Table 3. Multivariable adjusted risk analysis for having a TMSC category upgrade			
Variable	**OR**	**95% CI**	**p**
Age in years (1 unit increase)	0.95	0.87–1.03	0.202
Highest varicocele grade			
I	1		
II	0.53	0.11–2.53	0.426
III	1.01	0.21–4.93	0.988
Laterality			
Unilateral	1		
Bilateral	0.88	0.24–3.23	0.841
Testes volume (1 cc increase)	0.90	0.71–1.14	0.386
Preoperative TMSC (1 million units increase)	1.37	1.06–1.76	**0.015**
Preoperative FSH (1 IU/ml increase)	0.82	0.68–0.98	**0.026**
Preoperative testosterone (10 ng/dl increase)	1.02	0.98–1.05	0.368
Preoperative LH (1 IU/ml increase)	1.34	0.91–1.98	0.135

FSH: follicle-stimulating hormone; CI: confidence interval; LH: luteinizing hormone; OR: odds ratio; TMSC: total motile sperm count.

Table 4. Frequency of patients that had an upgrade of the TMSC category according to the FSH cutoff values and the positive likelihood ratio (+LR) of TMSC upgrade according to the different cutoff values of FSH in the overall patients		
FSH	**TMSC upgraded** n=33 (%)	**+LR of TMSC upgrade**
≤5 IU/ml	11 (33.3)	1.38
≤6 IU/ml	16 (48.8)	1.23
≤7 IU/ml	20 (60.6)	1.33
≤8 IU/ml	24 (72.7)	1.26
≤9 IU/ml	26 (78.8)	1.37
≤10 IU/ml	27 (81.8)	1.35
≤11 IU/ml	28 (84.8)	1.40
≤12 IU/ml	29 (87.9)	1.32
≤13 IU/ml	29 (87.9)	1.21
≤14 IU/ml	31 (93.9)	1.24
≤15 IU/ml	32 (97)	1.23
≤16 IU/ml	33 (100)	1.18

FSH: follicle-stimulating hormone; +LR: Positive likelihood ratio.