JOMH
Journal of Men's Health

Original Article
DOI: 10.22374/jomh.v15i3.143

ASSOCIATIONS BETWEEN PITUITARY-GONADAL AXIS PARAMETERS AND SEMEN QUALITY IN YOUNG, HEALTHY MEN (AndroLS)

Józków Paweł[1], Mędraś Marek[1,2], Szmigiero Leszek[3], Słowińska-Lisowska Małgorzata[1], Lwow Felicja[4]

[1]Department of Sports Medicine & Dietetics, University School of Physical Education, Wrocław, Poland

[2]Department of Endocrinology, Diabetology & Isotope Therapy, Wroclaw Medical University, Wrocław, Poland

[3]Department of Nucleic Acid Biochemistry, Medical University of Lodz, Łódź, Poland

[4]Department of Health Promotion, Faculty of Physiotherapy, University School of Physical Education, Wrocław, Poland

Corresponding author: Paweł Józków: jozkow@gmail.com

Submitted: 07 April 2019. Accepted: 04 June 2019. Published: 10 July 2019.

ABSTRACT

Background and Objective

Hormonal measurements play an important role in the evaluation of male fertility potential. In men without hypogonadism, the impact of androgen status on semen is rarely analyzed.

Material and Methods

We evaluated associations between parameters of the pituitary-gonadal axis: luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone (T), free testosterone (FT), calculated testosterone (TC), bioavailable testosterone (TB), sex hormone-binding globulin (SHBG), free androgen index, and semen quality parameters in healthy young men.

Results

In our study group, sperm concentration and sperm count were associated with FSH and FT. The percentage of immotile sperm was associated with LH and T. The percentage of vital sperm was negatively related to LH. We identified negative, independent associations between T and semen volume ($p<0.026$) and TC and semen volume ($p<0.025$). We observed negative, independent association between FSH, FT, and total sperm count (both $P<0.002$) and between FT and the percentage of normal forms ($P<0.012$). There were positive associations between LH and T and the percentage

of immotile sperm (P<0.007 and P<0.034, respectively). There were no relationships between sperm morphology parameters and the parameters of the pituitary-gonadal axis.

Conclusion

In healthy eugonadal men, variations in FSH, LH, T, and FT (within normal limits) are reflected in semen characteristics but not in sperm morphology features.

Key Words; *luteinizing hormone, follicle stimulating hormone, testosterone, semen quality, hormones*

INTRODUCTION

Semen analysis is a primary tool used to assess male fertility potential and is often combined with hormonal evaluations of the hypothalamo–pituitary–gonadal axis.[1]

The key reproductive hormones in men are follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T). Synthesis of FSH and LH in the pituitary gland is stimulated by gonadotropin-releasing hormone (GnRH). FSH interacts with Sertoli cells in the seminiferous tubules, and LH stimulates androgen synthesis in Leydig cells. Intratesticular testosterone (within the interstitial and seminal tubules) exerts multiple effects on spermatogenesis.[2]

Endocrine dysfunction, especially hypogonadism resulting from different causes, may affect semen quality and lead to reproductive failure. Increased FSH levels suggest disturbances of sperm production.[3,4] Associations between LH, T, and semen parameters are less clear. In some studies, T was not correlated with semen quality,[5,6] while in other studies, it was associated with sperm motility and morphology.[7,8] Associations between FT and sperm parameters have not been established.[9,10]

The presence of low T usually requires further evaluation because the precise assessment of androgen output is difficult in such circumstances.[11] The following measurements can be considered: free testosterone (FT), bioavailable testosterone (TB), TC, and sex-hormone binding globulin (SHBG) levels, as well as the free androgen index (FAI).[12] Unfortunately, most currently available assays for TT or FT are unsatisfactory.[13]

Questions on the significance of relationships between gonadotropin/androgens and semen parameters/sperm morphology are especially intriguing with regard to eugonadal men. Investigations in this specific group are few, and the scope of variations among hormonal/seminal constellations is considerable. Given the risk of potential bias,[14] a need for elucidating existing discrepancies has been indicated.[7]

Our aim was to evaluate the associations between pituitary–gonadal axis parameters and semen quality (including sperm morphology) in a sample of young healthy men.

METHODS

We performed our investigation in a homogenous group of young men (18–35 years) who studied or worked in the Lower Silesia region (Southern Poland, Europe). The project was approved by the Bioethics Committee of the University School of Physical Education in Wrocław (no. 36/2.12.2013), and we obtained written informed consent from all subjects before their participation. All the procedures were conducted in compliance with the Declaration of Helsinki for human subjects and the European Communities Council Directive of 24 November 1986 (86/609/EEC). The methodology of the AndroLS study was described in detail in our previous reports.[15–17]

From a target group of 5000 men, 500 subjects responded to the invitation and 300 were eligible for further analysis. Completed questionnaires and blood samples (for hormonal evaluations)

were acquired from 203 subjects. In total, semen samples were collected from 177 men. We excluded three subjects with no spermatozoa in the sperm samples and those with hormonal values beyond the normal range. The mean age of the studied subjects was 24.6±3.7 years, and their body mass index was 24.0±2.9 kg/m^2.

Hormonal Evaluation

Blood sampling was performed before 9.00 am after overnight fasting. Serum concentrations of FSH, LH, SHBG, total T, and FT were determined by electrochemiluminescence assay (ECLIA) using the Elecsys system COBAS E 601 (Roche, Switzerland). The intra- and interassay coefficients of variation (CVs) and the limits of detection were, respectively, as follows: 2.6%, 3.9%, and <0.100 mIU/mL for FSH; 0.9%, 1.9%, and 0.100 mIU/mL for LH; 1.4%, 2.7%, and 0.350 nmol/L for SHBG; 5.3%, 6.3%, and 0.025 ng/mL for total T; and 6.4%, 8.0%, and 0.002 pg/mL for FT. The FAI was calculated as testosterone/SHBG × 100. Calculated free testosterone (TC) and TB levels were calculated using measured serum concentrations of total testosterone and SHBG (at a fixed albumin concentration of 4.3 g/dL) using equations developed by the Hormonology Department, University Hospital of Ghent, Belgium (http://www.issam.ch/freetesto.htm).

Statistical Analysis

Continuous variables were first analyzed for normal distribution using the Kolmogorov–Smirnoff test with the Lilliefors correction test. Almost all semen parameters exhibited non-normal distributions. Biochemical parameters describing the hormonal status of the participants, such as LH, FSH, T, and SHGB, exhibited normal distributions, whereas FAI, TC, TF, and TB did not pass the normality test. Descriptive statistics are presented based on untransformed data. All values are expressed as means±standard deviation (SD) as well as medians and 5th and 95th percentiles. Linear simple and multiple regression analyses were performed to estimate the associations between semen quality parameters and hormonal parameters. Dependent variables, such as semen volume, sperm concentration, and total sperm count, were transformed using the Box Cox transformation to obtain a normally distributed data set. Semen characteristics expressed as percentages (total sperm motility, vitality, and the amount of normal sperm forms) were first converted into proportions and were then transformed by arcsin square root transformation. Factors with P-values <0.1 in linear simple regression analysis were included in linear multiple regression analyses. A P-value of <0.05 was considered significant. The data were analyzed using the SigmaPlot (Systat Software Inc., London, UK) statistics package, version 13, with the exception of Box-Cox transformation which was performed using Statistica version 12 (StatSoft, Krakow, Poland).

RESULTS

Parameters of the pituitary–gonadal axis in the studied population are presented in Table 1. The mean concentrations of LH, FSH, T, FT, and SHBG were within normal ranges for healthy adults.

The characteristics of the semen samples of the participants are presented in Table 2, and a detailed description of sperm morphology is presented in Table 3. The mean values of the majority of the semen parameters of the participants were above the lower World Health Organization (WHO) thresholds.[18] The only exception was the total motility of sperm, which was below the lower reference limit set at 40%.

Associations between LH, FSH, T, FT, TC, TB, FAI, and SHBG are presented in Table 4.

We observed significant correlations between LH and testosterone as well as between FT and

TABLE 1 Outcomes of the Laboratory Evaluation of the Participants (n=174)

Variable	Mean±SD	Median (95% CI)
LH (mIU/mL)	4.9±2.3	4.5 (1.6–11.8)
FSH (mIU/mL)	4.1±3.5	3.2 (1.0–10.2)
T (ng/mL)	6.0±2.1	5.9 (3.2–10.9)
FT (ng/L)	15.2±6.4	14.5 (6.7–27.6)
TC (ng/mL)	0.12±0.10	0.10 (0.06–0.30)
TB (ng/mL)	2.6±1.1	2.4 (1.3–4.7)
FAI (nmol/L)	56.6±19.5	54.2 (27.2–118.5)
SHBG (nmol/L)	39.9±17.0	37.7 (14.2–92.1)

CI = confidence interval; FAI = free androgen index; FSH = follicle-stimulating hormone; FT = free testosterone; LH = luteinizing hormone; T = total testosterone; TB = testosterone bioavailable; TC = testosterone calculated; SHBG = sex hormone-binding globulin; SD = standard deviation.

TABLE 2 Semen Parameters of the Participants (n=174)

Variable	Mean±SD	Median (95% CI)
Time of liquefaction (min)	27.4±9.2	25.0 (15.0–53.8)
Semen volume (mL)	3.34±2.2	3.0 (0.9–6.1)
pH	7.9±0.2	7.9 (7.7–8.4)
Leukocytes (10^6/mL)	0.11±0.27	0.0 (0.0–1.0)
Sperm concentration (10^6/mL)	59±45	50 (5–184)
Total sperm number (10^6/ejaculate)	171±131	147 (13–563)
Progressive motility (%)	44.8±15.8	43.0 (24.0–75.0)
Total motility (%)	35.6±14.0	37.0 (8.0–61.6)
Normal forms (%)	14.6±6.7	14.0 (2.2–28.8)
Vitality (%)	60.3±14.7	61.5 (30.5–81.0)

CI = confidence interval; SD = standard deviation.

TB (Table 4). FSH was negatively and significantly associated with FT, TB and the FAI. There was a positive significant association between FSH and SHBG (Table 4).

We applied a linear regression method to search for associations between semen parameters, sperm morphology, and hormonal variables (Appendix: Tables 1A and 2A). We observed significant associations between sperm concentration/sperm count and FSH, FT (Figure 1). The percentage of immotile sperm was positively associated with LH and testosterone levels, whereas the percentage of vital sperm was negatively correlated with LH only (Figure 2).

Because a range of associations were on the verge of statistical significance, we performed a multiple regression analysis to evaluate any potential, mutual effect of multiple factors (independent variables) on a specific parameter (dependent variables) (Table 5).

We observed negative, independent associations between T, TC, and semen volume ($P<0.026$ and $P<0.025$, respectively). There were negative, independent associations between FSH, FT, and total sperm count (both $P<0.002$) and between

TABLE 3 Sperm Morphology of the Participants (n=174)

Morphology characteristics	Mean±SD	Median (95% CI)
Normal forms (%)	14.6±6.7	14.0 (2.2–28.9)
Pathological forms (%)	84.8±8.8	86.0 (70.3–97.0)
Amorphous-headed sperm (%)	54.8±6.9	54.5 (42.2–70.0)
Round-headed sperm (%)	3.9±8.7	3.0 (0.0–10.8)
Tapered-headed sperm (%)	2.2±3.4	1.0 (0.0–12.7)
Double-headed sperm (%)	0.3±0.6	0.0 (0.0–2.0)
Microcephalus-headed sperm (%)	2.1±2.4	2.0 (0.0–8.0)
Macrocephalus-headed sperm (%)	2.9±2.2	3.0 (0.0–7.8)
Head with cytoplasmic droplets (%)	0.1±0.2	0.0 (0.0–1.0)
Vacuolated-headed sperm (%)	3.7±1.8	3.0 (1.0–7.7)
Abnormal middle-piece sperm (%)	7.6±4.1	7.0 (2.0–16.7)
Abnormally tailed sperm (%)	8.1±4.3	7.0 (2.0–17.0)

CI = confidence interval; SD = standard deviation.

TABLE 4 Associations between Hormonal Parameters. Upper and Lower Values Represent the Regression Coefficient (r) and Statistical Significance (P)

	LH (mIU/mL)	FSH (mIU/mL)	FAI (nmol/L)	SHGB (nmol/L)
T (ng/mL)	**0.217 0.010***	−0.030 0.696	**0.254 <0.001***	**0.514 <0.001***
FT (ng/L)	**0.169 0.045**	**−0.166 <0.001***	**0.474 <0.001***	0.023 0.760
TC (ng/mL)	−0.022 0.793	−0.133 0.080	**0.288 <0.001***	−0.080 0.294
TB (ng/mL)	**0.167 0.047***	**−0.163 0.032***	**0.817 <0.001***	**−0.202 0.008***
FAI (nmol/L)	0.067 0.428	**−0.189 0.025***	–	–
SHGB (nmol/L)	0.082 0.336	**0.197 0.019***	**−0.639 <0.001***	–

FAI = free androgen index; FSH = follicle-stimulating hormone; FT = free testosterone; LH = luteinizing hormone; T = total testosterone; TB = testosterone bioavailable; TC = testosterone calculated; SHBG = sex hormone-binding globulin.
**p< 0.05.*

FT and the percentage of normal forms (P<0.012). In turn, we noted a positive association between LH, T, and the percentage of immotile sperm. The power of the test was ≥0.8.

In the multivariate regression analysis model, there were no significant relationships between sperm morphology characteristics and the hormonal milieu of the studied subjects.

DISCUSSION

We detected associations between the serum concentrations of gonadotropins/androgens and a number of semen quality parameters in young healthy men. We emphasize that such relationships were present in subjects with an intact pituitary–gonadal axis. We also found correlations between gonadotropins and free/bioavailable testosterone. We did not notice any significant associations between hormonal status and specific sperm morphology features in the studied group.

Testosterone, in concert with gonadotropins, enables intact spermatogenesis. LH stimulates the synthesis of testosterone in Leydig cells, whereas FSH is closely related to Sertoli cell

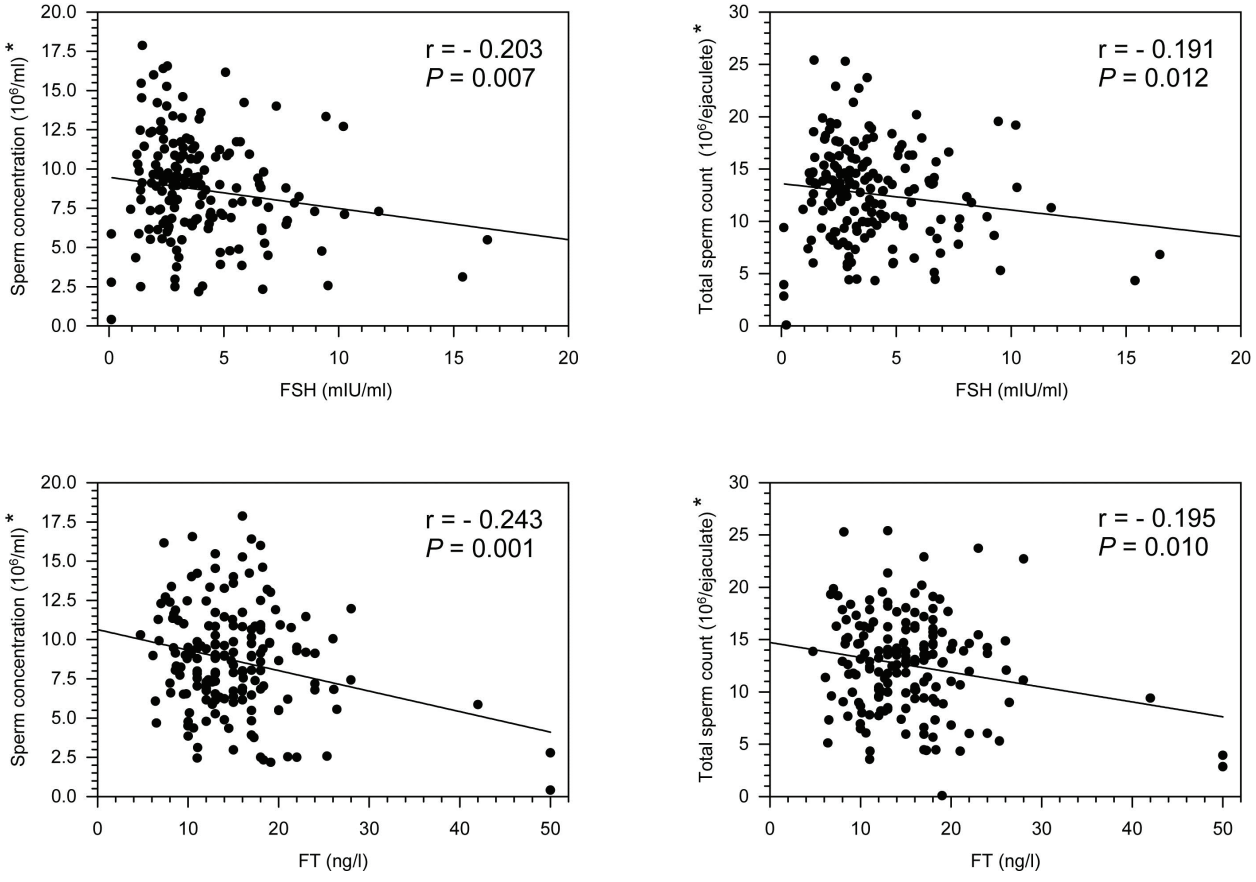

FIG. 1 Associations between follicle-stimulating hormone (FSH)/free testosterone (FT) concentrations and sperm concentration/total sperm count. *Data after Cox–Box transformation are shown. Data of semen characteristics were transformed to normality before regression analyses. λ values were in the range of 0.34 to 0.39, and all data sets passed the normality test after transformation.

function. Androgen signaling is essential for sperm production, and even transient alterations in testosterone concentrations (e.g., as the result of doping with anabolic-androgenic steroids) may impair or stop this process. Such actions occur within the mechanism of a negative impact loop with the pituitary (LH, FSH) and the hypothalamus (GnRH).[2] It is important to remember that blood concentrations do not parallel intratesticular concentrations of testosterone.

A few association studies performed in healthy men have suggested an inverse relationship between gonadotropins and semen quality; for example, in a study of healthy Americans, a negative correlation was detected between FSH (evaluated by RIA or a bio-assay) and sperm count, motility, and normal sperm.[5] A similar observation (with regard to sperm concentration and count) was made in Australian [3] and (with regard to sperm count and sperm morphology) in Flemish subjects.[9] In many studies, the quality of semen was related to LH, although in healthy subjects from another region within our country, this relationship was not observed.[19]

As expected, LH was correlated with T, FT, and TB in our study subjects. Similar relationships between LH and testosterone evaluations were

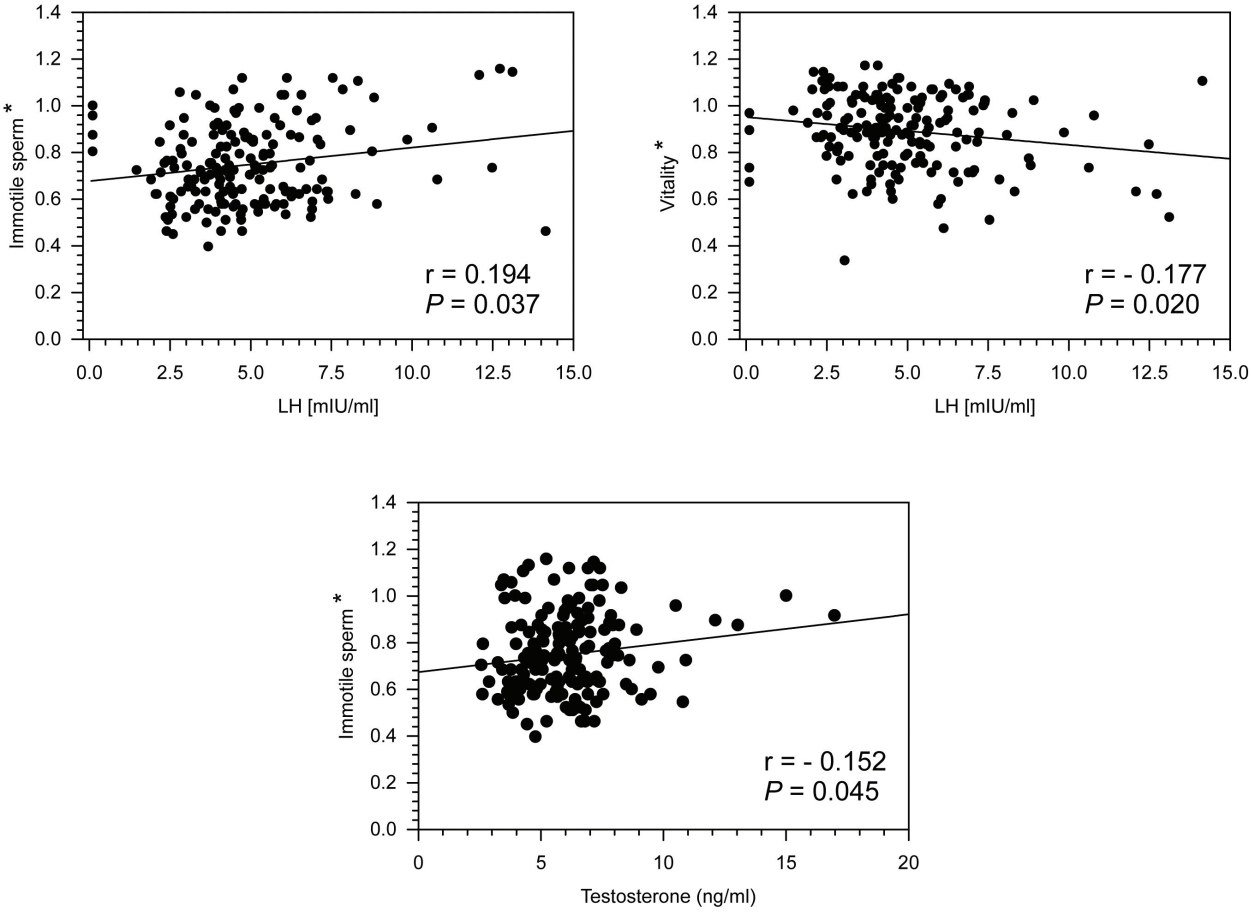

FIG. 2 Associations between luteinizing hormone (LH)/testosterone concentrations and the percentages of immotile and vital sperm. *Semen characteristics were normalized by arcsin square root transformation to pass the normality test.

demonstrated previously.[5,14] LH is often thought to be related to spermatozoa motility; however, according to our observations, higher concentrations of LH (and T) were associated with higher percentages of immotile and less vital sperm. We attempt to explain this based on a hypothesis involving compensated Leydig cell dysfunction.[14] According to the abovementioned phenomenon, normal testosterone concentration is secured by an increase in LH release; then, sperm production is assumed to be maintained at a level that is sufficient for reproduction. Research suggesting the presence of worrisome trends in semen quality, sperm counts, and other reproductive problems (e.g., the deterioration of Leydig cell function) further supports this hypothesis.[20]

Despite their potential clinical value, variations of normal concentrations of LH and T are not analyzed as an indicator of altered spermatogenesis. However, it is clear that higher than normal concentrations of LH are detected in infertile men.[8]

FSH is necessary for optimal sperm production; however, its role is thought to be primarily synergistic, and spermatogenesis may be maintained in FSH-deficient subjects.[2,21] In men of Danish couples who had not previously attempted to achieve a pregnancy, there was a trend toward

TABLE 5 The Results of Multiple Regression Analysis for Associations between Semen Quality and Morphology Characteristics and the Levels of Testosterone and Gonadotropins

Dependent variable: semen volume (mL)			
	β	***P***	**α**
T (ng/mL)	**−0.175**	**0.026***	0.801
TC (ng/mL)	**−0.177**	**0.025***	
Dependent variable: sperm concentration (10^6/mL)			
LH (mIU/mL)	−0.090	0.251	0.999
FSH (mIU/mL)	**−0.227**	**0.005***	
T (ng/mL)	0.067	0.498	
FT (ng/L)	**−0.297**	**0.002***	
TB (ng/mL)	−0.060	0.560	
Dependent variable: total sperm count (10^6/ejaculate)			
FSH (mIU/mL)	**−0.230**	**0.002***	0.980
FT (ng/L)	**−0.233**	**0.002***	
Dependent variable: normal forms (%)			
FT (ng/L)	**−0.215**	**0.012***	0.866
FAI (nmol/L)	−0.031	0.712	
FT (ng/L)	**−0.202**	**0.008***	
TB (ng/mL)	**−0.196**	**0.010***	
Dependent variable: immotile sperm (%)			
LH (mIU/mL)	**0.205**	**0.007***	0.932
T (ng/mL)	**0.154**	**0.034***	
FT (ng/L)	0.099	0.280	

FAI = free androgen index; FSH = follicle-stimulating hormone; FT = free testosterone; LH = luteinizing hormone; T = total testosterone; TB = testosterone bioavailable; TC = testosterone calculated; SHBG = = sex hormone-binding globulin; β = standardized regression coefficient; α = power of the test.
**p< 0.05.*

higher sperm concentrations in subjects with lower FSH.[4] Consistent with this, reduced sperm concentration has been associated with increased FSH in oligospermic men.[22] It has also been reported that isolated FSH deficiency is associated with a low percentage of motile sperm.[23] In the literature, several cases of an autosomal recessive disease manifesting as azoospermia have been described in the presence of normal testosterone levels; these cases were caused by mutations of the FSH beta-subunit.[24]

In our participants, FSH was negatively associated with FT, TB, and FAI and positively associated with SHBG. Interestingly, we observed that high-normal concentrations of FSH were associated with higher sperm count and sperm concentration in this group. We hypothesize that the differences in semen quality may be present even within the normal limits for FSH. Similar to the abovementioned changes of Leydig cell action, increased production of FSH might compensate for non-overt alterations in sperm production. For example, it has been documented that body mass index is associated with semen parameters, and slimmer men were found to have higher FSH levels.[25] It has also been suggested

that the administration of FSH might be effective in infertile patients with normal FSH levels. Whether FSH treatment in such cases positively affects the structure of the spermatozoon, DNA fragmentation, oxidative processes, or aneuploidy (which might, e.g., reflect polymorphisms of the FSH receptor gene) remains hypothetical.[26]

Thus far, there is no gold standard for total T measurement in the clinical setting. All available assays have substantial shortcomings.[13,27] In our investigation, T was correlated with semen volume only. According to other reports, total T was found to be related to the motility of spermatozoa.[25] The relationship between LH/T and immotile sperm observed by us was not reported in healthy volunteers. We found a negative association between T, TC, and semen volume. We could not find any information on such relationships in studies by other authors.

We found (in our linear regression analysis) that FT was inversely related to sperm concentration, total sperm count, and the percentage of normal sperm. These findings suggest a potential role for this parameter in evaluating semen quality. We are aware of the methodological problems encountered during FT evaluation. Unfortunately, due to the study design, we were unable to investigate the relationship between serum and seminal concentrations of FT, which can associate differently with semen parameters.[10]

In our analysis, none of the parameters describing sperm morphology were related to hormonal markers. Previously, it was suggested that FSH concentrations may be negatively correlated with the percentage of normal sperm.[4] Similar relationships were also reported for infertile men.[28] This area of seminal diagnostics is relatively poorly understood.

A strength of our study is that the data were gathered from a unique group that has no special interest in medical (particularly andrological) investigations. Young healthy men do not undergo routine andrological screening unless faced with fertility issues. The study subjects were derived from a genetically homogenous population. We avoided potential biases resulting from the collection of semen at home, seasonal fluctuations of andrological parameters, and interobserver inconsistencies/variation. All semen samples were collected during one season (winter) in a room adjacent to a laboratory that specializes in andrological evaluations. The samples were assessed by a single, experienced medical analyst. The performance of the laboratory is routinely verified by an international, external quality control program. Additionally, we tested the significance of potential lifestyle confounders (diet, alcohol consumption, physical activity, and mobile phone usage).

Among the limitations of our investigation, we have to include the analyses of only one semen sample and one blood sample per subject. Recent evidence suggests that at least in cases of semen samples, analysis of a single sample can be sufficient.[29] Of note, our volunteers were not compensated for their participation. We did not measure inhibin B because this parameter is not routinely used in clinical practice. In our previous publications, we discussed the possible impacts of physical activity[15] and alcohol consumption[16] on semen parameters in the studied group. With regard to the latter, the findings are conflicting.[30] The educational level of the participants was above average: all had accomplished at least 12 years of education. The participants lived in an industrialized region of Poland, which can be compared to other regions in Europe. We are aware of the geographical and even regional variations in semen characteristics.[31] We questioned our subjects on maternal tobacco smoking and on the use of other substances/drugs, but the responses did not correlate with semen or hormonal characteristics (data not shown). A detailed analysis of prenatal or current chemical/toxic exposures was beyond the scope of this study.

Our results pertain to young urban subjects not seeking help for fertility. We would refrain from simply transferring these results to the general population or subfertile/infertile men and

those preparing for ART (assisted reproductive technology) procedures. The findings might have been different if we had included subjects with a previous medical history, chronic diseases, hypogonadal issues, etc.

CONCLUSIONS

In healthy young men with untested fecundity, concentrations of gonadotropins are correlated with concentrations of bioavailable and free testosterone, and semen quality is significantly associated with variations in pituitary–gonadal axis parameters.

Differences in concentrations of FSH, LH, T, and FT (all within normal limits) seem to be reflected in semen characteristics.

An in-depth evaluation of the hormonal milieu may have significance for the assessment of semen quality in subjects with hyper- or hypogonadism as well as in eugonadal men.

CONFLICT OF INTERESTS

The authors have declared that they have no conflicts of interest.

FUNDING

This study was financially supported by the University School of Physical Education, Wrocław, Poland.

REFERENCES

1. Skakkebæk NE, Giwercman A, de Kretser D. Pathogenesis and management of male infertility. Lancet 1994;343:1473–9. https://doi.org/10.1016/S0140-6736(94)92586-0
2. O'Donnell L, Stanton P, de Kretser DM. Endocrinology of the Male Reproductive System and Spermatogenesis. In: LJ De Groot, G Chrousos, K Dungan, KR Feingold, A Grossman, JM Hershman et al. (eds.), Endotext [Internet]. South Dartmouth, MA;MDText.com, Inc.; 2000. Available from http://www.ncbi.nlm.nih.gov/books/NBK279031/. PubMed PMID: 25905260.
3. Stewart TM, Liu DY, Garrett C, Jorgensen N, Brown EH, Baker HW. Associations between andrological measures, hormones and semen quality in fertile Australian men: inverse relationship between obesity and sperm output. Hum Reprod 2009;24:1561–8. https://doi.org/10.1093/humrep/dep075
4. Jensen TK, Andersson AM, Hjollund NH, et al. Inhibin B as a serum marker of spermatogenesis: correlation to differences in sperm concentration and follicle-stimulating hormone levels. A study of 349 Danish men. J Clin Endocrinol Metab 1997;82:4059–63. https://doi.org/10.1210/jc.82.12.4059
5. Uhler ML, Zinaman MJ, Brown CC, Clegg ED. Relationship between sperm characteristics and hormonal parameters in normal couples. Fertil Steril 2003;79 Suppl 3:1535–42. https://doi.org/10.1016/S0015-0282(03)00336-4
6. Sina D, Schuhmann R, Abraham R, Taubert HD, Dericks-Tan JS. Increased serum FSH levels correlated with low and high sperm counts in male infertile patients. Andrologia 1975;7:31–7. https://doi.org/10.1111/j.1439-0272.1975.tb01223.x
7. Keskin MZ, Budak S, Zeyrek T, et al. The relationship between serum hormone levels (follicle-stimulating hormone, luteinizing hormone, total testosterone) and semen parameters. Arch Ital Urol Androl 2015;87:194–7. https://doi.org/10.4081/aiua.2015.3.194
8. Andersson AM, Jorgensen N, Frydelund-Larsen L, Rajpert-De Meyts E, Skakkebaek NE. Impaired Leydig cell function in infertile men: a study of 357 idiopathic infertile men and 318 proven fertile controls. J Clin Endocrinol Metab 2004;89:3161–7. https://doi.org/10.1210/jc.2003-031786
9. Dhooge W, van Larebeke N, Comhaire F, Kaufman JM. Regional variations in semen quality of community-dwelling young men from Flanders are not paralleled by hormonal indices of testicular function. J Androl 2007;28:435–43. https://doi.org/10.2164/jandrol.106.001644
10. Wang G, Xu R, Zhang Z, Wang X. [Detection of free testosterone in the serum and semen of idiopathic oligospermia patients and its significance]. Zhonghua Nan Ke Xue 2004;10:684–5.

11. Wang C, Catlin DH, Demers LM, Starcevic B, Swerdloff RS. Measurement of total serum testosterone in adult men: comparison of current laboratory methods versus liquid chromatography-tandem mass spectrometry. J Clin Endocrinol Metab 2004;89:534–43. https://doi.org/10.1210/jc.2003-031287
12. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84:3666–72. https://doi.org/10.1210/jcem.84.10.6079
13. Rosner W, Auchus RJ, Azziz R, Sluss PM, Raff H. Utility, limitations, and pitfalls in measuring testosterone: an endocrine society position statement. J Clin Endocrinol Metab 2007;92:405–13. https://doi.org/10.1210/jc.2006-1864
14. Jorgensen N, Joensen UN, Toppari J, et al. Compensated reduction in Leydig cell function is associated with lower semen quality variables: a study of 8182 European young men. Hum Reprod 2016;31:947–57. https://doi.org/10.1093/humrep/dew021
15. Jozkow P, Medras M, Lwow F, Zagrodna A, Slowinska-Lisowska M. Associations between physical activity and semen quality in young healthy men. Fertil Steril 2017;107:373–8.e2. https://doi.org/10.1016/j.fertnstert.2016.11.004
16. Lwow F, Medras M, Słowińska-Lisowska M, Jóźków P, Szmigiero L. The effect of occasional alcohol drinking on semen quality and sperm morphology among young and healthy polish men. J Mens Health 2017;13:e16–24. https://doi.org/10.22374/1875-6859.13.2.3
17. Mędraś M, Lwow F, Jóźków P, Szmigiero L, Zagrodna A, Zagocka E, Słowińska-Lisowska M. The quality of semen among a sample of young, healthy men from Lower Silesia (AndroLS). Endokrynol Pol 2017;68(6):668–675. doi: 10.5603/EP.a2017.0056. Epub 2017 Oct 12. PubMed PMID: 29022649.
18. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva, Switzerland: World Health Organization; 2010.
19. Kamieniczna M, Fraczek M, Malcher A, et al. Semen quality, hormonal levels, and androgen receptor gene polymorphisms in a population of young male volunteers from two different regions of Poland. Med Sci Monit 2015;21:2494–504. https://doi.org/10.12659/MSM.893628
20. Skakkebaek NE, Rajpert-De Meyts E, Buck Louis GM, et al. Male Reproductive disorders and fertility trends: influences of environment and genetic susceptibility. Physiol Rev 2016;96:55–97. https://doi.org/10.1152/physrev.00017.2015
21. Conway GS. Clinical manifestations of genetic defects affecting gonadotrophins and their receptors. Clin Endocrinol (Oxf). 1996;45:657–63. https://doi.org/10.1046/j.1365-2265.1996.8680879.x
22. Subhan F, Tahir F, Ahmad R, Khan ZD. Oligospermia and its relation with hormonal profile. J Pak Med Assoc 1995;45:246–7.
23. Salama N, El-Sawy M. Isolated low follicle stimulating hormone (FSH) in infertile males – a preliminary report. Arch Ital Urol Androl 2013;85:118–24. https://doi.org/10.4081/aiua.2013.3.118
24. Zheng J, Mao J, Cui M, et al. Novel FSHbeta mutation in a male patient with isolated FSH deficiency and infertility. Eur J Med Genet 2017;60:335–9. https://doi.org/10.1016/j.ejmg.2017.04.004
25. Jensen TK, Andersson AM, Jorgensen N, et al. Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. Fertil Steril 2004;82:863–70. https://doi.org/10.1016/j.fertnstert.2004.03.056
26. Valenti D, La Vignera S, Condorelli RA, et al. Follicle-stimulating hormone treatment in normogonadotropic infertile men. Nat Rev Urol 2013;10:55–62. https://doi.org/10.1038/nrurol.2012.234
27. Herati AS, Cengiz C, Lamb DJ. Assays of serum testosterone. Urol Clin North Am 2016;43:177–84. https://doi.org/10.1016/j.ucl.2016.01.003
28. Kumanov P, Nandipati K, Tomova A, Agarwal A. Inhibin B is a better marker of spermatogenesis than other hormones in the evaluation of male factor infertility. Fertil Steril 2006;86:332–8. https://doi.org/10.1016/j.fertnstert.2006.01.022
29. Chiu YH, Edifor R, Rosner BA, et al. What does a single semen sample tell you? Implications for

male factor infertility research. Am J Epidemiol 2017;186:918–26. https://doi.org/10.1093/aje/kwx169

30. Jensen TK, Swan S, Jorgensen N, et al. Alcohol and male reproductive health: a cross-sectional study of 8344 healthy men from Europe and the USA. Hum Reprod 2014;29:1801–9. https://doi.org/10.1093/humrep/deu118
31. Jorgensen N, Andersen AG, Eustache F, et al. Regional differences in semen quality in Europe. Hum Reprod 2001;16:1012–9. https://doi.org/10.1093/humrep/16.5.1012

APPENDIX

There are two additional tables available for interested readers: Tables 1A and 2A.

TABLE 1A Correlation coefficients by simple regression analyses between semen quality parameters and various hormonal characteristics (n=174).

	Semen volume (ml)	Semen pH	Sperm concentration (10^6/ml)	Total sperm count (10^6/ ejaculate)	Progressive motility (%)	Total motility (%)	Immotile (%)	Vitality (%)
LH (mIU/ml)	-0.030 0.692	**0.205** **0.007***	-0.131 0.087	-0.093 0.222	0.006 0.940	**-0.194** **0.011***	**0.194** **0.037***	**-0.177** **0.020***
FSH (mIU/ml)	-0.055 0.470	0.048 0.529	**-0.203** **0.007***	**-0.191** **0.012***	0.120 0.149	0.006 0.938	0.012 0.870	-0.002 0.977
T (ng/ml)	-0.126 0.098	0.062 0.419	-0.128 0.092	-0.011 0.888	0.051 0.541	-0.110 0.151	**0.152** **0.045***	-0.101 0.187
FT (ng/l)	-0.110 0.150	0.114 0.136	**-0.243** **0.001***	**-0.195** **0.010***	0.050 0.544	-0.081 0.288	0.128 0.094	-0.044 0.568
TC (ng/ml)	-0.128 0.093	0.038 0.621	-0.125 0.101	0.019 0.799	-0.017 0.818	-0.016 0.833	0.047 0.540	-0.011 0.883
TB (ng/ml)	0.043 0.573	0.032 0.672	**-0.152** **0.045***	-0.060 0.430	-0.092 0.266	-0.011 0.887	0.103 0.179	0.080 0.296
FAI (nmol/l)	0.083 0.277	0.027 0.721	-0.112 0.143	-0.009 0.907	-0.004 0.964	0.002 0.979	0.016 0.830	0.029 0.706
SHBG (nmol/l)	-0.004 0.961	0.142 0.062	0.009 0.962	-0.001 0.988	0.076 0.362	-0.059 0.444	0.072 0.349	-0.076 0.322

Upper and lower values represent the regression coefficient (r) and statistical significance (P).
LH, luteinizing hormone; FSH, follicle-stimulating hormone; T, total testosterone; FT, free testosterone; TC, testosterone calculated; TB, testosterone bioavailable; FAI, free androgen index; SHBG, sex hormone-binding globulin.

TABLE 2A Correlation coefficients by simple regression analyses between sperm morphology quality parameters and various hormonal characteristics.

	Normal forms (%)	**Pathological forms (%)**	**Amorphous head (%)**	**Round head (%)**	**Tapered head (%)**	**Microcephalus head (%)**	**Macrocephalus head (%)**	**Vacuolated head (%)**	**Abnormal middle-piece (%)**	**Abnormal tail (%)**
LH (mIU/ml)	-0.054	0.067	0.119	-0.056	0.078	0.053	-0.031	0.008	-0.094	-0.072
	0.479	0.381	0.120	0.464	0.306	0.487	0.685	0.919	0.220	0.346
FSH (mIU/ml)	-0.031	0.055	0.033	0.002	0.092	0.027	0.115	0.149	-0.102	-0.137
	0.686	0.474	0.667	0.976	0.229	0.724	0.132	0.050	0.182	0.073
T (ng/ml)	-0.094	0.080	-0.027	0.028	-0.119	0.067	0.146	0.029	0.114	0.085
	0.218	0.295	0.725	0.717	0.120	0.381	0.055	0.704	0.134	0.265
FT (ng/l)	**-0.230**	**0.190***	-0.036	0.001	-0.088	-0.010	0.043	-0.003	**0.262***	0.105
	0.002*	**0.012**	0.936	0.986	0.248	0.893	0.572	0.967	**<0.001**	0.170
TC (ng/ml)	-0.081	0.065	0.021	0.063	-0.113	-0.096	**0.152***	0.012	0.027	-0.003
	0.287	0.683	0.783	0.410	0.140	0.211	**0.046**	0.867	0.727	0.971
TB (ng/ml)	-0.055	0.071	0.049	0.010	-0.099	-0.087	0.065	-0.050	0.123	0.099
	0.468	0.352	0.518	0.897	0.195	0.253	0.394	0.516	0.108	0.195
FAI (nmol/l)	-0.103	0.051	0.101	-0.026	-0.026	-0.064	0.065	-0.105	0.088	0.069
	0.081	0.502	0.185	0.734	0.734	0.400	0.394	0.170	0.246	0.367
SHBG (nmol/l)	0.047	0.006	0.107	-0.035	-0.035	0.017	0.125	0.043	-0. 011	0.051
	0.543	0.932	0.159	0.265	0.650	0.821	0.100	0.573	0.888	0.506

Upper and lower values represent the regression coefficient (r) and statistical significance (P). LH, luteinizing hormone; FSH, follicle-stimulating hormone; T, total testosterone; FT, free testosterone; TC, testosterone calculated; TB, testosterone bioavailable; FAI, free androgen index; SHBG, sex hormone-binding globulin.