Single random measurement of urinary gonadotropin concentration for screening and monitoring of girls with central precocious puberty

Running title: Urinary gonadotropin in central precocious puberty

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Abstract

Purpose: The gold standard for assessing pubertal activation is the gonadotropin-releasing hormone (GnRH) stimulation test (GnRHST), which is invasive, time-consuming and inconvenient. This study evaluated whether a single random measurement of urinary luteinizing hormone (LH) concentration could substitute for the GnRHST in diagnosing and monitoring central precocious puberty (CPP) in girls.

Methods: Fifty-five girls with breast buds before 8 years of age were assessed by both the GnRHST and urinary gonadotropin assays. Based on GnRHST results, 29 girls were assigned to the CPP group (peak LH ≥ 5 IU/L) and 26 to the premature thelarche (PT) group (peak LH < 5 IU/L). Auxological data and urine and serum samples were collected at baseline and after treatment with GnRH agonist for 12 and 24 weeks.

Results: Although auxological data did not differ between the two groups, the levels of insulin-like growth factor-1, basal LH and follicle stimulating hormone (FSH), estradiol, peak LH, urinary LH, and peak LH/FSH and urinary LH/FSH ratios were higher in the CPP than in the PT group. Pearson’s correlation analysis showed a positive correlation between urinary and serum LH concentration ($r = 0.660$, $P < 0.001$). Receiver-operating characteristic curve analyses showed that urinary LH concentration 0.725 IU/L was a cutoff significantly reflecting positivity on the GnRHST. Urinary LH and FSH concentrations declined significantly during GnRH agonist treatment.

Conclusion: A single, random measurement of urinary gonadotropin concentration may be reliable for the initial screening and monitoring of CPP in girls.

Key words: precocious puberty, gonadotropins, urine, gonadotropin-releasing hormone
Introduction

The initiation of puberty requires an increase in the pulsatile release from the hypothalamus of gonadotropin-releasing hormone (GnRH), which activates the pulse generation of the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the anterior pituitary. This is followed by gonadal growth, sex steroid production, and gametogenesis. Because the concentrations of LH and FSH increase about 50- to 100-fold and about 10-fold, respectively, between childhood and adulthood, the serum concentrations of basal gonadotropins, especially LH, may reflect secretion occurring during pubertal development. However, radioimmunoassay measurements of basal gonadotropin concentrations have been shown to be inaccurate, because some concentrations are below the detectable limit and because of variations in the diurnal levels of these hormones during the prepubertal and early pubertal periods.

Precocious puberty (PP) is historically defined as the onset of developmental signs of sexual maturation before age 8 years in girls and 9 years in boys. The three major subtypes of PP are central PP (CPP), peripheral PP, and benign pubertal variants, such as premature thelarche (PT), premature adrenarche, premature pubarche, and gynecomastia. The most common form, CPP, is caused by premature maturation of the hypothalamic-pituitary-gonadal (HPG) axis.

The GnRH stimulation test (GnRHST) is considered the gold standard for identifying pubertal activation of the HPG axis and for diagnosing CPP. GnRH agonists (GnRHa), which suppress the HPG axis, have been shown to be effective and safe for the treatment of patients with CPP. The therapeutic effects of GnRHa can be monitored by measuring gonadotropin responses using the GnRHST. However, performance of the GnRHST involves a long stay in a hospital and repeated blood sampling by venipuncture. Because this testing is expensive,
invasive, and inconvenient for children with early signs of puberty, studies have evaluated methods that can replace or simplify the standard GnRHST.5, 8-9)

Recent technological improvements in hormone assays have increased interest in using urine or saliva samples to assess human pituitary function. The collection and analysis of urine samples would be more convenient and better tolerated in special situations, such as in neonates and children with needle phobia, and for long-term studies. Urinary gonadotropin concentrations have been shown to distinguish among pubertal stages.10-11) For example, LH concentrations in the first morning-voided (FMV) urine and in 24 h urine have been shown to correlate with basal serum LH concentration.5, 12) In addition, CPP can be assessed and monitored by measuring LH concentration and LH/FSH ratio in FMV urine samples. However, FMV urine sampling and 24 h urine collection are also inconvenient in outpatient settings.

To determine whether measuring gonadotropin concentrations in single, random urinary samples can be used as an initial screening for diagnosing CPP and for monitoring the response of girls with CPP to treatment with GnRHa, the present study compared the concentrations of urinary gonadotropins and of basal and peak serum gonadotropins in girls with CPP and PT.

Materials and methods

1. Participants

The study protocols were approved by the Institutional Review Board of the Chungnam National University Hospital, Daejeon, South Korea, and written informed consent was obtained from all participating patients and their legitimate guardians (IRB No. 2020-09-095).
Girls showing signs of early puberty and who had undergone both the GnRHST and urinary gonadotropin assays were recruited from the growth clinic in the Department of Pediatrics, Chungnam National University Hospital, from August 2017 to July 2018. CPP was defined as breast budding as a first sign of puberty before 8 years of age accompanied by an at least 1-year greater bone age than chronological age and a peak LH level ≥ 5.0 IU/L during the rapid-acting GnRHST (0.1 mg Relefact LHRH; Sanofi-Aventis, Frankfurt am Main, Germany). PT was defined as a peak LH < 5.0 IU/L in the GnRHST. Subjects were excluded if they had an underlying disorder or history of medication associated with altered pubertal timing or gonadal development, such as steroids, growth hormone, or GnRHa, or if they were found to have an abnormality on brain MRI or pelvis sonography.

2. Study design
The age, height, weight, body mass index (BMI), parental height, pubertal status, bone age, thyroid function, concentrations of insulin-like growth factor-1 (IGF-1) and estradiol, and medical history were collected from the patients’ medical records. The standard deviations (SDs) for height, weight, and BMI were calculated using the 2017 growth reference for Korean children and adolescents. Venous blood and random urine samples were obtained from all participants before and 15, 30, 45, 60, and 90 min after GnRH injection. Blood and urine samples were collected from girls with CPP after three and six injections of GnRHa (50–100 µg/kg, leuprolide acetate; Takeda, Osaka, Japan).

3. Gonadotropin analysis
The concentrations of serum and urinary LH and FSH were measured using a two-site sandwich immunoassay involving a direct chemiluminometric technique on an ADVIA Centaur instrument (Siemens Healthcare Diagnostics Inc., New York, NY, USA). The LH
assay had a range of 0.07–200 mIU/mL, with an interassay correlation coefficient of 0.99 and a total coefficient of variation (CV) of 2.7–3.8%. The FSH assay had a range of 0.3–200 mIU/mL, an interassay correlation coefficient of 0.99, and a total CV of 2.2–3.9%.

4. Statistics
Continuous variables are presented as means ± SDs. The presence of a normal distribution was determined using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The means of auxological and clinical laboratory data in the CPP and PT groups were compared by independent t tests. Relationships between serum and urinary variables and positive results on the GnRHST were determined using Pearson’s bivariate correlation, receiver-operating characteristic (ROC) curve, and logistic regression analyses. Associations between serum and urinary LH concentrations were evaluated by linear regression analysis. Mean serum and urinary LH levels in response to GnRHa treatment were compared by paired-sample t tests. Graphs were drawn using GraphPad Prism 6 for Windows (GraphPad Software Inc., San Diego, CA, USA). Data were analyzed using IBM SPSS Statistics for Windows (version 22; IBM Corp, Armonk, NY, USA), with P < 0.05 considered statistically significant.

Results
1. Clinical and laboratory characteristics
Of the 55 girls who showed signs of early puberty and who had undergone both the GnRHST and urinary gonadotropin assays, 29 were assigned to the CPP group (peak LH ≥ 5 IU/L) and 26 to the PT group (peak LH < 5 IU/L). Their anthropometric and laboratory characteristics are summarized in Table 1. Age, height, weight, parental height, predicted adult height, bone age, Tanner stage, and urinary gonadotropin/creatinine (Cr) ratio did not differ significantly
between the two groups. However, IGF-1, estradiol, basal serum LH and FSH, and peak serum LH concentrations were significantly higher in the CPP than in the PT group ($P < 0.05$ each). In addition, peak serum LH/FSH ratio in the GnRHST, and basal urinary LH and LH/FSH ratio were significantly higher in the CPP group ($P < 0.05$ each).

2. **Association between serum gonadotropin and urinary gonadotropin levels**

The associations between pairs of urinary and serum variables obtained in the GnRHST were calculated using Pearson’s bivariate correlation analysis (Table 2). Urinary LH concentration correlated strongly and positively with urinary LH/FSH ratio ($r = 0.884$, $P < 0.001$, data not shown), basal serum LH ($r = 0.660$, $P < 0.001$) and FSH ($r = 0.618$, $P < 0.001$) levels, urinary FSH level ($r = 0.523$, $P < 0.001$), peak serum LH level ($r = 0.280$, $P = 0.017$), peak serum LH/FSH ratio ($r = 0.471$, $P = 0.001$, not shown), and IGF-1 level ($r = 0.295$, $P = 0.044$, data not shown). Urinary LH concentration, however, did not correlate significantly with peak serum FSH level ($r = 0.168$, $P = 0.255$).

3. **Urinary gonadotropin levels as predictors of a positive GnRHST**

To determine whether measuring urinary LH level can be used for the diagnosis of CPP, ROC curves were determined, and the areas under the ROC curves (AUCs) calculated (Table 3). The AUCs for urinary LH ($0.721 \pm 0.077$, $P = 0.010$) and serum LH ($0.749 \pm 0.072$, $P = 0.003$) concentrations for a positive GnRHST result (peak LH $\geq 5.0$ IU/L) were similar. The peak serum LH/FSH ratio had the highest sensitivity and specificity for diagnosing CPP ($0.958 \pm 0.030$, $P < 0.001$), and the urinary LH/FSH ratio was also significant ($0.731 \pm 0.075$, $P = 0.007$). The cutoff of urinary LH level for a positive GnRHST was 0.725 IU/L (65.4% sensitivity and 63.6% specificity), which represents a fourfold improvement in the prediction
of a positive GnRHST (odds ratio [OR], 4.048; 95% confidence interval [CI], 1.210–13.538; 
\(P = 0.023\)) by logistic regression analysis. The cutoff for the urinary LH/FSH ratio was 0.057, 
which had a sensitivity of 65.4% and a specificity of 68.2% (Fig. 1).

4. Changes in urinary gonadotropin levels in girls with CPP after treatment with 
GnRHa

Treatment of girls with CPP with GnRHa for 12 and 24 weeks resulted in significant 
reductions from baseline in serum and urinary LH and FSH concentrations (Fig. 2).

Discussion

Urinary concentrations of gonadotropins can be easily, noninvasively, and conveniently 
measured, suggesting that they may be a good substitute for a positive GnRHST in girls with 
CPP. The present study found that randomly measured urinary concentrations of 
gonadotropins strongly correlated with the serum concentrations of gonadotropins, and that 
urinary gonadotropins were predictive of a positive GnRHST, suggesting that this method 
may be useful for monitoring girls with CPP during GnRHa treatment.

Although the GnRHST is the gold standard for the diagnosis of CPP, this method has 
disadvantages when used in outpatient clinics because it is time-consuming, expensive, 
invasive, and labor intensive for both patients and physicians. Previous studies have tested 
methods to reduce the discomfort and provide a more convenient way to diagnose CPP. A 
study tested the efficacy of a single measurement of unstimulated LH level \(\geq 0.83\) IU/L for 
the diagnosis of CPP, but that study did not test the efficacy of the LH/FSH ratio.\(^{14}\) These 
assays, however, have shown limitations that the basal serum gonadotropin levels may 
overlap between prepubertal and pubertal children.\(^{15}\) A single blood sample obtained 40 min
after the injection of GnRH (gonadorelin) was found useful for the diagnosis of CPP, with 98% sensitivity and 100% specificity, whereas a sample obtained 20 min after injection was useful for the assessment of pubertal suppression, with 100% sensitivity and specificity.\textsuperscript{16} A simplified GnRHST involving a single sample obtained 30 min after GnRH injection was found useful for the diagnosis of CPP and for assessment of its suppression.\textsuperscript{17, 18} Because the unstimulated serum LH levels in the present study accounted for 74.9% of the positivity of the test for CPP, we thought that a single measurement of basal serum LH concentration would be better for initial screening of puberty onset, not for the diagnosing CPP instead of GnRHST.

Urine sampling has advantages over blood sampling, because the former is convenient, painless, and more tolerable for young patients. In the 1960s, some scientists detected the urinary excretion of LH and FSH in adults and reported the usefulness of urine samples for the differentiation of sex hormone disorders.\textsuperscript{19-20} Ultrasensitive liquid chromatography–tandem mass spectrometry (LC-MS/MS)-based steroid assays can detect and measure low concentrations of estradiol, testosterone, and LH in the serum and urine of peripubertal and pubertal children.\textsuperscript{21-22}

Gonadotropins can be measured in 24 h urine samples obtained from healthy prepubertal children, but this method is cumbersome, requiring many technical procedures and much effort.\textsuperscript{23} A single, nontimed measurement of urinary gonadotropin levels was reported to be effective in children, with urinary LH/FSH ratios in pubertal children being significantly higher than serum LH/FSH ratios in prepubertal children.\textsuperscript{10} Urinary LH/Cr ratios were also found to be higher in pubertal than in prepubertal boys, but not in girls, whereas urinary FSH/Cr ratios were higher in pubertal than in prepubertal girls, but not in boys. The authors commented that the lack of significance of the LH/Cr ratio in girls likely reflected the random
nature of the urine collection and the episodic excretion of gonadotropins, with urinary FSH excretion being more variable in both the prepubertal and pubertal groups. In another study of both boys and girls, urinary LH/Cr and LH/FSH ratios could be used to distinguish between children with CPP and prepubertal children or those with other early pubertal disorders, whereas urinary FSH/Cr ratios contributed to the detection of gonadal failure, but not CPP.

Our results are consistent with those of the previous studies. We found that urinary LH levels and urinary LH/FSH ratios, but not urinary FSH/Cr ratios, differed significantly between girls with CPP and PT girls. We did not evaluate Cr-adjusted urinary concentrations because this adjustment may result in over- or under-estimation of urinary LH and FSH concentrations. Similar to previous studies, we found that urinary gonadotropin concentrations correlated strongly with serum gonadotropin levels without adjustment for Cr. However, urinary LH/Cr and FSH/Cr ratios were not positively correlated with their serum basal and peak levels. Singh et al. demonstrated that the steroid and LH concentrations in FMV urine samples in adolescents are not significantly influenced by hydration status and may not require adjustment for Cr or specific gravity. Similarly, we also found that these adjustments did not strengthen the correlations between urinary gonadotropin concentrations and paired basal serum gonadotropin levels.

FMV urine samples are better indicators of gonadotropin excretion because they avoid nocturnal episodic variation. The use of FMV urine to monitor pubertal changes was introduced over a decade ago. Assays of LH concentration in FMV urine were found to be more sensitive in distinguishing rapid progressive PP from slow progressive PP than measurements of GnRH-stimulated gonadotropins (peak LH/FSH ratio and peak FSH level).

Interestingly, urinary concentrations of bisphenol A adequately reflected the average
exposure, regardless of the source of urine, whether spot urine, FMV, or 24 h collection, suggesting that the use of single spot urine samples may be reliable for measuring urinary bisphenol A concentrations.\(^{28}\)

To date, no consensus has been reached about monitoring the effects of treatment in patients with CPP, other than assessing physical changes and bone age. HPG suppression is usually evaluated by measuring GnRH-stimulated or unstimulated serum gonadotropin and sex hormone concentrations.\(^{29}\) We evaluated the ability of a single random urine sample to evaluate HPG suppression in girls after GnRHa treatment. The measurements of spot urinary LH and FSH levels before and during GnRHa treatment reflected the response to HPG suppression as accurately as did the single serum gonadotropins levels. LH concentration in FMV urine can also be used to monitor pubertal suppression after GnRHa treatment in patients with CPP.\(^{29}\) However, in the outpatient clinic, FMV urine collection is inconvenient for patients. Our strategy is a more convenient method and appears to be as effective as other tests.

The present study had several limitations. The sample size was small, and we did not evaluate the changes in urinary gonadotropin concentrations after the girls stopped taking GnRHa.

In conclusion, this study describes a reliable and convenient method for the initial screening and monitoring of young patients with signs of early puberty. The single random urinary LH concentration and urinary LH/FSH ratio were strong noninvasive reflectors of a positive GnRHST. Urinary LH and FSH levels may be useful markers for monitoring HPG suppression during GnRHa treatment of children with CPP. Longer-term studies with more participants are needed to determine the optimal strategies and guidance for obtaining urine samples to assess hormone status in children and adolescents.
Conflicts of interest

No potential conflict of interest relevant to this article was reported.
References


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25. Lucaccioni L, McNeilly J, Mason A, GiacomoZZi C, Kyriakou A, Shaikh MG, et al. The measurement of urinary gonadotropins for assessment and management of pubertal...


Figure Legends

Fig. 1. ROC curves for a positive GnRH stimulation test. A) Urinary LH level, B) urinary LH/FSH ratio, C) serum basal LH level.
Abbreviations: ROC, receiver operating characteristics; GnRH: gonadotropin-releasing hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone

Fig. 2. Changes in serum and urinary gonadotropin levels in girls with CPP from before to after treatment with GnRHa. (A1) Urinary LH level, (A2) urinary FSH level. (B1) serum LH level, (B2) serum FSH level. The values are presented as mean ± SD.
Abbreviations: CPP: central precocious puberty, GnRHa: gonadotropin-releasing hormone agonist; LH, luteinizing hormone; FSH, follicle-stimulating hormone; SD: standard deviation
Table 1. Auxological and clinical characteristics of the participants

<table>
<thead>
<tr>
<th></th>
<th>Central Precocious Puberty (N = 29)</th>
<th>Premature thelarche (N = 26)</th>
<th>P value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>8.2 ± 0.6</td>
<td>8.1 ± 0.7</td>
<td>0.558</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>131.7 ± 4.6</td>
<td>129.8 ± 5.2</td>
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</tr>
<tr>
<td>Weight (Kg)</td>
<td>30.3 ± 3.8</td>
<td>30.855 ± 5.9</td>
<td>0.747</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>17.4 ± 1.52</td>
<td>18.1 ± 2.5</td>
<td>0.256</td>
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<tr>
<td>Height SDS</td>
<td>1.01 ± 0.76</td>
<td>0.81 ± 0.76</td>
<td>0.355</td>
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<tr>
<td>Weight SDS</td>
<td>0.91 ± 0.96</td>
<td>0.86 ± 0.79</td>
<td>0.828</td>
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<tr>
<td>BMI SDS</td>
<td>0.45 ± 0.66</td>
<td>1.0 ± 1.9</td>
<td>0.173</td>
</tr>
<tr>
<td>MPH (cm)</td>
<td>160.5 ± 3.9</td>
<td>160.6 ± 3.7</td>
<td>0.934</td>
</tr>
<tr>
<td>PAH (cm)</td>
<td>150.2 ± 5.5</td>
<td>151.0 ± 3.9</td>
<td>0.566</td>
</tr>
<tr>
<td>MPH-PAH</td>
<td>10.3 ± 5.4</td>
<td>9.6 ± 4.3</td>
<td>0.625</td>
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<tr>
<td>Bone age (yr)</td>
<td>10.4 ± 0.9</td>
<td>9.9 ± 0.8</td>
<td>0.059</td>
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<td>BA-CA (yr)</td>
<td>2.2 ± 0.8</td>
<td>1.8 ± 0.8</td>
<td>0.087</td>
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<td>Tanner stage</td>
<td>2.2 ± 0.4</td>
<td>2.1 ± 0.3</td>
<td>0.375</td>
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<td>IGF-1 (ng/mL)</td>
<td>272.5 ± 96.2</td>
<td>222.4 ± 33.4</td>
<td>0.021</td>
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<tr>
<td>Basal LH (IU/L)</td>
<td>1.2 ± 0.7</td>
<td>1.0 ± 0.5</td>
<td>0.005</td>
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<td>Basal FSH (IU/L)</td>
<td>2.1 ± 1.2</td>
<td>1.9 ± 1.7</td>
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<td>Estradiol (pg/mL)</td>
<td>22.1 ± 10.5</td>
<td>15.2 ± 8.1</td>
<td>0.018</td>
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<td>Peak LH (IU/L)</td>
<td>12.3 ± 7.9</td>
<td>3.0 ± 1.1</td>
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<td>Peak FSH (IU/L)</td>
<td>9.5 ± 3.2</td>
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<td>Peak LH/FSH ratio</td>
<td>1.3 ± 0.6</td>
<td>0.37 ± 0.2</td>
<td>&lt;0.001</td>
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<tr>
<td>Test</td>
<td>Mean ± SD 1</td>
<td>Mean ± SD 2</td>
<td>p-value</td>
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<tr>
<td>Urine LH (IU/L)</td>
<td>1.2 ± 0.6</td>
<td>0.7 ± 0.3</td>
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<td>Urine FSH (IU/L)</td>
<td>14.6 ± 3.4</td>
<td>13.4 ± 2.3</td>
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<tr>
<td>Urine Cr. (mg/dL)</td>
<td>113.5 ± 56.6</td>
<td>93.1 ± 51.1</td>
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<tr>
<td>Urine LH/FSH ratio</td>
<td>0.08 ± 0.03</td>
<td>0.05 ± 0.02</td>
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<td>Urine LH/Cr.</td>
<td>0.0135 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.577</td>
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<td>Urine FSH/Cr.</td>
<td>0.19 ± 0.2</td>
<td>0.24 ± 0.03</td>
<td>0.508</td>
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</table>

Abbreviations: BMI, body mass index; MPH, mean parenteral height; PAH, predicted adult height; BA-CA, bone age minus chronological age; IGF-1, insulin like growth factor-1; LH, luteinizing hormone; FSH, follicle stimulating hormone; Cr., creatinine.

All variables were presented as mean ± standard deviation.

* These statistics were analyzed by independent *t* test, and *P* <0.05 was statistically significant.
Table 2. Correlations between urinary and serum gonadotropin levels

<table>
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<tr>
<th></th>
<th>bLH</th>
<th>bFSH</th>
<th>pLH</th>
<th>pFSH</th>
<th>uLH</th>
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<tbody>
<tr>
<td>bFSH</td>
<td>0.622**</td>
<td></td>
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<tr>
<td>pLH</td>
<td></td>
<td>0.644**</td>
<td>0.464**</td>
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<tr>
<td>pFSH</td>
<td>0.057</td>
<td>0.158</td>
<td>0.383**</td>
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<tr>
<td>uLH</td>
<td>0.660**</td>
<td>0.618**</td>
<td>0.280*</td>
<td>0.168</td>
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<td>uFSH</td>
<td>0.543*</td>
<td>0.625**</td>
<td>0.192</td>
<td>0.088</td>
<td>0.523**</td>
</tr>
</tbody>
</table>

Abbreviations: bLH, basal serum luteinizing hormone; bFSH, basal serum follicle-stimulating hormone; pLH, peak serum luteinizing hormone on GnRH stimulation test; pFSH, peak serum follicle-stimulating hormone on GnRH stimulation test; uLH, urine luteinizing hormone; uFSH, urine follicle-stimulating hormone.

All variables were calculated by Pearson’s bivariate correlations analysis, and significance were presented as * <0.05 and ** <0.01.
Table 3. Area under the ROC curve for a positive GnRH stimulation test

<table>
<thead>
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<th>Variables</th>
<th>Area</th>
<th>SE</th>
<th>P value</th>
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</thead>
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<td>Peak serum LH (≥5 IU/L)</td>
<td>1.000</td>
<td>0.000</td>
<td>&lt;0.001</td>
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<td>Peak serum LH/FSH ratio</td>
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<td>0.030</td>
<td>&lt;0.001</td>
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<tr>
<td>Basal serum LH (IU/L)</td>
<td>0.749</td>
<td>0.072</td>
<td>0.003</td>
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<tr>
<td>Urine LH/FSH ratio</td>
<td>0.731</td>
<td>0.075</td>
<td>0.007</td>
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<tr>
<td>Urine LH (IU/L)</td>
<td>0.721</td>
<td>0.077</td>
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Abbreviations: ROC, receiver operating characteristics; SE, standard error; LH, luteinizing hormone; FSH, follicle-stimulating hormone