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REVIEW ARTICLE

Urine Gonadotropin Measurement in Laboratory for Evaluating the Function of HPGA in Girls

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SUMMARY

Background: **The evaluation of hypothalamic-pituitary-gonadal axis function is essential for girls with pubertal disorders. The laboratory gold standard for evaluating the axis is blood gonadotropin level during gonadotropin-releasing hormone stimulation test. However, these tests need venipuncture and repeated blood collection, which affect the compliance of children and parents.**
Methods: **Studies were conducted on the basis of a computer-assisted search of the literature published in English using the National Library of Medicine, PubMed, Google Scholar, and Google databases, and published in Chinese core journals.**
Results: **According to this review, urine collection is non-invasive and convenient. Urine gonadotropin can reflect the average level of blood, which can reflect the HPGA function of girls with pubertal disorders. However, because of the limited sensitivity of LH detection, urine Gn during the GnRH stimulation test cannot replace that of the blood.**
Conclusions: **It is worth improving the sensitivity of LH detection kits. In the future, perhaps most exciting is replacing blood for evaluating HPGA function in girls with the urine Gn determination in the lab during the GnRH stimulation test.**
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KEY WORDS

urine gonadotropin measurement, lab, function, hypothalamic-pituitary-gonadal axis, girl

LIST OF ABBREVIATIONS

HPGA - Hypothalamus-Pituitary-Gonadal Axis
CPP - Central Precocious Puberty
GnRH - Gonadotropin-Releasing Hormone
Gn - Gonadotropin
RIA - Radioimmunoassay
IRMA - Immunoradiometric Assay
IFMA - Immunofluorometric Assay
ICMA - Immunochemiluminometric Assay
GnRHa - Gonadotropin-Releasing Hormone Analogs
ROC - Receiver Operating Characteristics
AUC - Area Under the Receiver Operating Characteristics Curve

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INTRODUCTION

In recent years, girls with puberty developmental disorders (such as precocious puberty, etc.) are increasing. In these diseases, it is essential to evaluate the function of the hypothalamus-pituitary-gonadal axis (HPGA). At present, the laboratory gold standard for diagnosing central precocious puberty (CPP) is the gonadotropin releasing hormone (GnRH) stimulation test. It often requires tedious processes, repeated punctures and multiple blood collections, which affect the compliance of children and parents. Urine collection is non-invasive and convenient, and the urine gonadotropin (Gn) can reflect the average blood Gn level. With the development of Gn detection technology, the potential clinical application value of urine Gn in girls with endocrine-related diseases is gradually increasing.

MATERIALS AND METHODS

Articles were collected on the basis of a computer-assisted search of the literature published in English using the National Library of Medicine, PubMed, Google Scholar, and Google databases, and published in Chinese core journals. The following terms were used to search independently and in combination: urine gonadotropin measurement, lab, function, hypothalamic-pituitary-gonadal axis, and girl.

The development of urine Gn detection assays

A sensitive, reliable, and convenient detection assay is an indispensable tool for urine Gn detection. Until now, the detection assays of urine Gn have gone through three generations.

The first generation of urine Gn detection technique, biological assay, originated in the 1940s. Urine Gn was detected in girls over four years and four months old, a milestone in history. However, this biological assay often requires tedious and time-consuming steps, such as urine extraction and acidification. Limited detection sensitivity and high labor intensity make it challenging to apply in clinical practice [1].

The second-generation assays include radioimmunoassay (RIA) and immunoradiometric assay (IRMA). Both techniques originated in the 1970s when double-antibody RIA was used to detect urine Gn [2]. The urine luteinizing hormone (LH) and follicle-stimulating hormone (FSH) detection limits are 2.6 IU/L and 1.3 IU/L, respectively [3]. These methods suggest insufficient sensitivity to detect the elevated urine LH level in prepuberty or early puberty. Besides, the urine components still need to be extracted and concentrated for RIA. The urine sample needs to be incubated for around a week for double-antibody RIA and also needs to be incubated for 3 hours at 37°C or 20 hours at 20°C for simplified solid-phase RIA. They both depend on the treatment of radioactive substances [4]. Subsequently, IRMA is used in urine Gn detection research. Although the sensitivity of urine Gn detection has been improved, it is radioactive. It has also not been widely used in clinics [5]. The third generation of urine Gn detection techniques, immunofluorometric assay (IFMA) and immunochemiluminometric assay (ICMA), originated in the 1980s [6]. It can detect low prepubertal urine Gn and morning urine Gn to evaluate the efficacy of gonadotropin releasing hormone analogs (GnRHa) in children [7]. IFMA could detect the peak level of urine LH, while IRMA could not [8]. The detection limits of LH and FSH in the urine by ICMA are both 0.1 IU/L. The detection limits of LH and FSH in the urine by IFMA are 0.6 IU/L and 1.0 IU/L, respectively [9]. Compared with IFMA, ICMA may be more sensitive and accurate in detecting the reference range of children's urine Gn, which has less overlap between early puberty and prepuberty, and uses few reagents [9,10]. Our team's research shows that ICMA can be used to detect urine Gn in the range of normal pH (4.6 - 8.0). Urine LH is more sensitive to long-term preservation and multiple freezing and thawing than urine FSH [11], which further confirms the reliability of ICMA detection of urine Gn in children.

The urine Gn determination in the evaluation of HPGA function in girls

At present, the laboratory gold standard for evaluating children's HPGA function is still the GnRH stimulation test, which often requires tedious processes such as repeated punctures and repeated blood collection, which affects the compliance of children and their parents. In recent years, many clinicians or researchers have tried to simplify the procedures of these tests or look for alternative methods such as urine Gn detection to evaluate the function of HPGA in girls.

Detection of the spontaneous blood Gn level in the evaluation of HPGA function in girls

The spontaneous blood Gn (IFMA) level can be useful in screening the HPGA activation in children. Several studies suggested the level of LH in spontaneous blood can reflect the process of HPGA initiation better than that of FSH. The LH level and its pulse amplitude increase from early prepuberty to pubertal onset were more than FSH [12]. Mean prepubertal LH was 0.04 +/- 0.04 IU/L, rising 100-fold during puberty. Spontaneous FSH levels were much higher than LH values, and rose at puberty [13]. However, because the secretion of blood LH (ICMA) has a pulsatile or circadian pattern, it has limited value for a single spontaneous blood LH level in the daytime to evaluate the function of HPGA [14]. For example, in girls with early stages of puberty, the spontaneous blood LH (ICMA) before 10:00 in the morning is more sensitive than that after 10:00 or in the afternoon [15]. Among girls with very low spontaneous blood LH (ICMA) levels, more than 36% were diagnosed with CPP [16]. The elevated spontaneous blood LH (IFMA) level was highly predictive of the HPGA initiation, while a low LH level did not exclude HPGA

initiation [17]. In our team's previous study, there was no significant difference ($p > 0.05$) in diurnal spontaneous blood LH and FSH levels between CPP and non-CPP girls. There was no correlation between diurnal spontaneous blood LH and peak LH during the GnRHa stimulation test in CPP girls ($p > 0.05$). The diurnal spontaneous blood LH ≥ 0.3 IU/L was present in 78.8% of CPP. When diurnal spontaneous blood LH was ≥ 0.3 IU/L, 80.8% of girls suffered from CPP, compared with 42.9% of non-CPP. When diurnal spontaneous blood LH was ≥ 0.7 IU/L and nocturnal spontaneous blood LH ≥ 1.1 IU/L, 100% of girls suffered from CPP. The CPP girls with peak LH between 5 - 8 IU/L did not have both diurnal spontaneous blood LH ≥ 0.7 IU/L and nocturnal spontaneous blood LH ≥ 1.1 IU/L. It indicates that the spontaneous blood LH measured by ICMA cannot be used as a screening tool for CPP. So, the detection of spontaneous blood LH levels could not replace blood Gn after the GnRH stimulation test.

Detection of the blood Gn level during GnRH or GnRHa stimulation test

The blood Gn level (ICMA) during the GnRH test is the gold standard for HPGA initiation [18]. In girls with the later puberty phase (Tanner stages IV - V), spontaneous blood LH levels (ICMA) usually elevate, while spontaneous blood LH levels in girls in the early puberty phase (Tanner stages II - III) often overlap with those of prepubertal girls (Tanner stage I) [19]. A monthly GnRH stimulation test is required when CPP is suspected, but spontaneous blood LH levels (ICMA) are within the prepuberty range [20]. An ICMA GnRH-stimulated LH level greater than 5 IU/L suggests maturing gonadotropin secretion [13]. Traditional GnRH stimulation test (ICMA) requires repeated blood collection at different time points, and the duration is usually 90 to 120 minutes, affecting the compliance of children and parents [21]. Many clinicians and researchers have tried to simplify these tests, hoping that the Gn level in a single blood sample can evaluate children's HPGA function. Studies have shown that Gn (ICMA/IRMA) in a single blood sample at 30 or 45 minutes after stimulation is as effective an index in diagnosing CPP as the traditional GnRH stimulation test [22].

Detection of the spontaneous urine Gn level

The spontaneous urine Gn level is more valuable in screening the initiation of HPGA in children than spontaneous blood LH. The urine Gn level can reflect the average blood Gn level within a period.

The spontaneous urine LH (ICMA) level strongly correlated with the blood LH peak after the GnRH stimulation test [23]. In our team study of girls with CPP, the spontaneous nocturnal urine and spontaneous blood LH level detected by ICMA were highly correlated ($r = 0.546$, $p < 0.001$). The spontaneous nocturnal urine and peak blood LH detected by ICMA were highly correlated ($r = 0.597$, $p < 0.001$). The spontaneous diurnal and peak blood LH had a similar correlation ($r = 0.514$, $p < 0.001$). It suggested that spontaneous nocturnal urine Gn correlated well with spontaneous and stimulated blood Gn. The correlation between the spontaneous nocturnal urine and peak blood LH level is not lower than it is between the spontaneous diurnal and peak blood LH. In addition, we used the receiver operating characteristics (ROC) analysis to evaluate the diagnostic value of CPP. When spontaneous nocturnal urine LH > 0.113 IU, the sensitivity and specificity of diagnosing CPP were 71.4% and 90.5%, respectively, and the area under the ROC curve (AUC) was 0.825; when nocturnal spontaneous blood LH > 1.39 IU/L, the sensitivity and specificity for diagnosing CPP were 54.8% and 85.7%, respectively, and the AUC was 0.654; when diurnal spontaneous blood LH > 0.97 IU/L, the sensitivity and specificity of diagnosing CPP were 42.5% and 90.5%, respectively, and the AUC was 0.680. Thus, spontaneous nocturnal urine LH level has the value for CPP screening, which may be superior to that of spontaneous blood LH.

The urine Gn level (RIA) in the morning can reflect children's 24h Gn secretion in early puberty [24]. The urine Gn (ICMA) level in normal girls aged 0 - 6 years old decreased with the increase of age, urine LH/creatinine (Cr) slowly reduced from 2 years old, and urine FSH/Cr slowly reduced from 5 years old [25]. When the detection limit of both urine LH and FSH by ICMA was 0.20 U/L, the detection rate of urine LH in normal preschool girls (11.54%) was further lower than that of FSH (99.04%). The detection rate of urine LH in obese preschool girls (15.57%) was also lower than that of FSH (98.39%), mainly due to the low level of urine LH in preschool girls. In a large proportion of prepubertal girls, the sensitivity of the available ICMA assay was not yet able to detect urine LH. Urine FSH levels were higher in girls aged 3 and 4 years than in those of 5 and 6 - 7 years old. Our previous study indicated that preschool girls still have mini-puberty at 3 to 4 years of age. During the development of puberty, the level of spontaneous urine LH (IFMA) was higher than that in the blood. The urine LH level in girls increased from prepubertal 0.07 IU/L to 11-year-old 2.0 IU/L, 12-year-old 5.0 IU/L, 14-year-old 6.3 IU/ L, and blood LH level increased from early puberty 0.04 IU/L to late puberty 3.7 IU/L. The urine LH level in girls began to increase at about 9 years old, about 2 years earlier than that of blood. In girls under 8 years old, the urine LH level was lower than 0.5 IU/L [26].

In our team study, the application of ICMA to detect nocturnal 12 hours urine gonadotropin may be helpful in evaluating the initiation of HPGA in children. When spontaneous urine LH/Cr > 68.806 IU/mol, the sensitivity and specificity for diagnosing CPP were 71.8% and 79.3%, respectively, and AUC was 0.790; when spontaneous urine LH/FSH > 0.1253, the sensitivity and specificity of CPP were 51.3% and 89.7%, respectively, and AUC was 0.713; when spontaneous blood LH > 1.08 IU/L, the sensitivity and specificity for diagnosing CPP were 53.8% and 96.6%, respectively, and AUC

was 0.762; when spontaneous blood LH/FSH > 0.1634, the sensitivity and specificity of diagnosing CPP were 71.8% and 58.6%, respectively, and AUC was 0.634. Nocturnal urine LH/Cr and LH/FSH values are not lower than that of blood LH and LH/FSH [27]. We combined diurnal urine Gn (ICMA) and nocturnal urine Gn to evaluate the function of HPGA in 52 children. The specificity and sensitivity of HPGA initiation were 100% and 88.5%, respectively, when the diurnal urine LH content was ≥ 0.036 IU and the nocturnal urine LH content was ≥ 0.068 IU. The specificity and sensitivity of HPGA initiation were 96.2% and 84.6%, respectively, when the diurnal urine LH/Cr ≥ 20.7230 IU/mol and the nocturnal urine LH/Cr ≥ 34.1691 IU/mol. In evaluating the initiation of HPGA, the value of combined detection of diurnal urine Gn may be better than its single index [28].

The urine LH (ICMA) level can distinguish between rapidly and slowly progressive precocious puberty in girls. The morning LH level of girls with rapidly progressive precocious puberty was higher than that with slowly progressive precocious puberty and prepubertal girls [29]. The AUC of LH (IFMA) and LH/FSH in the morning could predict puberty of girls with Tanner stage, which was better than those of the GnRH stimulation test. The specificities of urine LH level and LH/FSH were 96% and 79% in the morning, respectively, while the specificity of the GnRH stimulation test was 66%. It is suggested that the detection of morning urine Gn may be better than the detection of blood Gn after the GnRH stimulation test in the evaluation of HPGA initiation in girls [30].

A strong correlation has been confirmed between the spontaneous urine LH level (IFMA) and the blood LH level after GnRH stimulation in girls with GnRHa treatment [31]. In 25 girls with CPP, the morning urine LH (IRMA) levels were suppressed to a range matched with age after a 12-week GnRHa treatment [32]. In a study of 56 girls with CPP treated with GnRHa 3 - 4 times, spontaneous morning urine Gn (ICMA) was confirmed to be in accordance with the inhibition of HPGA. The results showed that the sensitivity of morning urine LH level to monitor the efficacy of GnRHa was almost the same as that of the GnRH (or GnRHa) stimulation test; when morning urine LH was 1.01 IU/L, the sensitivity of evaluating HPGA inhibition was 92.3%, and the specificity was 100% [31]. It can be seen that morning urine Gn can be useful in monitoring the efficacy of GnRHa and evaluating the inhibition of HPGA.

Therefore, the morning urine LH level can be used as a screening tool for HPGA initiation or inhibition. However, the spontaneous urine Gn (ICMA) level detection, as the spontaneous blood Gn level detection, still cannot replace the blood Gn level detection during the GnRH stimulation test [33].

Detection of the urine Gn level during GnRH or GnRHa stimulation test

Our team's study showed that the urine Gn (ICMA) detection rate during the triptorelin stimulation test was significantly higher than the spontaneous urine and blood Gn. The urine Gn level within 24 hours after the triptorelin stimulation test is useful for evaluating girls' HPGA function. Our study revealed that the correlations between blood peak LH and stimulated urine LH/Cr (from the first 12-hour and the second 12-hour urine) were higher than those between blood peak LH and the third overnight or the fourth overnight or spontaneous urine LH/Cr [34].

We collected the urine of 50 children with pubertal disorders in five consecutive segments at 1.5 hours, 2.5 hours, 3.5 hours, 4.5 hours, and 5.5 hours during the triptorelin stimulation test. The AUC for urine LH, LH/Cr, and LH/FSH evaluating HPGA initiation at the 4.5 hours period were 0.958, 0.955, and 0.908. The first two areas reached the highest values of all periods of urine, and the latter was very close to the highest value of 0.910. It suggested that the 4.5 hours time urine Gn reflected the function of HPGA, and its value was not lower than that of 5.5 hours. When stimulated 4.5 hours urine LH ≥ 0.2893 IU/L, the sensitivity and specificity were 92.6% and 91.3%, respectively. When spontaneous blood LH ≥ 0.7950 IU/L, the sensitivity and specificity were 66.7% and 87%, respectively. So, the stimulated urine LH was superior to spontaneous blood LH in evaluating HPGA initiation. Therefore, 4.5 hours urine Gn level can reflect the function of HPGA in the GnRHa stimulation test [35]. The ratio of urine Gn level of 3.5 hours during triptorelin stimulation test or GnRHa stimulation test and the diurnal spontaneous urine Gn detected by ICMA may be useful in evaluating the efficacy of GnRHa in children. The value of urine FSH level and its ratio to diurnal spontaneous urine FSH level may be more useful. When the 3.5 hours urine LH ≤ 0.083 IU and FSH ≤ 1.089 IU, and when the blood peak LH ≤ 2.30 IU/L and peak FSH ≤ 2.39 IU/L, the specificities were all 100%. The sensitivities of 3.5 hours urine LH and FSH were 90.91% and 100%, and the sensitivities of blood peak LH and FSH were 95.45% and 100%, respectively. It can be seen that from the perspective of the sensitivity and specificity of judging the efficacy of GnRHa, it is suggested that the non-invasive urine Gn detection during the GnRHa simulated test can also reflect the blood Gn peak during GnRHa treatment, and the urine FSH value may be higher than the urine LH [36]. Therefore, during the GnRHa stimulation test, urine Gn can better reflect the HPGA function than spontaneous urine Gn in girls.

CONCLUSION

Urine collection is non-invasive and convenient. Urine gonadotropin can reflect the average level of blood, which can reflect the HPGA function of girls with pu-

bertal disorders. However, because of the limited sensitivity of LH detection, urine Gn during the GnRH stimulation test cannot replace that of the blood. Better yet, urine collection is non-invasive and convenient. It is worthwhile to improve the sensitivity of LH detection kits. In the future, perhaps the most exciting development is urine Gn determination in the lab during the GnRH stimulation test which can replace that of blood for evaluating the HPGA function in girls.

Declaration:
We declare that this review paper is our original work.

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Declaration of Interest:
All authors declared that there are no financial or non-financial competing interests.

References:

1. Catchpole HR, Greulich WW. The excretion of gonadotrophic hormone by prepuberal and adolescent girls. J Clin Invest 1943; 22(6):799-804. (PMID: 16695064)

2. Chipman JJ, Moore RJ, Marks JF, et al. Interrelationship of plasma and urinary gonadotropins: correlations for 24 hours, for sleep/wake periods, and for 3 hours after luteinizing hormone-releasing hormone stimulation. J Clin Endocrinol Metab 1981;52 (2):225-30. (PMID: 6780585)

3. Kulin HE, Bell PM, Santen RJ, Ferber AJ. Integration of pulsatile gonadotropin secretion by timed urinary measurements: an accurate and sensitive 3-hour test. J Clin Endocrinol Metab 1975;40(5):783-9. (PMID: 1173241)

4. Tamada T, Tsukui Y. [Measurement of urinary LH by a new solid phase radioimmunoassay]. Nihon Naibunpi Gakkai Zasshi 1971; 47(3):174-80. (PMID: 5105282)

5. le Cotonnec JY, Porchet HC, Beltrami V, Khan A, Toon S, Rowland M. Clinical pharmacology of recombinant human follicle-stimulating hormone (FSH). I. Comparative pharmacokinetics with urinary human FSH. Fertil Steril 1994;61(4):669-78. (PMID: 8150109)

6. Woodhead JS, Weeks I. Magic Lite design and development. J Biolumin Chemilumin 1989;4:611-4. (PMID: 2678929)

7. Roth CL, Brendel L, Rückert C, Hartmann K. Antagonistic and agonistic GnRH analogue treatment of precocious puberty: tracking gonadotropin concentrations in urine. Horm Res 2005;63(5): 257-62. (PMID: 15995342)

8. Kesner JS, Knecht EA, Krieg EF Jr, Wilcox AJ, O'Connor JF. Detecting pre-ovulatory luteinizing hormone surges in urine. Hum Reprod 1998;13(1):15-21. (PMID: 9512221)

9. Resende EA, Lara BH, Reis JD, Ferreira BP, Pereira GA, Borges MF. Assessment of basal and gonadotropin-releasing hormone-stimulated gonadotropins by immunochemiluminometric and immunofluorometric assays in normal children. J Clin Endocrinol Metab 2007;92(4):1424-9. (PMID: 17284632)

10. Singh GK, Jimenez M, Newman R, Handelsman DJ. Immunoreactive LH in long-term frozen human urine samples. Drug Test Anal 2014;6(4):336-41. (PMID: 23606665)

11. Ma Y, Xu Z, Gu G, Ren F, Jin H, Zhu W. Effects of storage conditions on urinary LH and FSH measurement using immunochemiluminometric assay. Clin Lab 2018;64(5):877-82. (PMID: 29739050)

12. Mitamura R, Yano K, Suzuki N, Ito Y, Makita Y, Okuno A. Diurnal rhythms of luteinizing hormone, follicle-stimulating hormone, testosterone, and estradiol secretion before the onset of female puberty in short children. J Clin Endocrinol Metab 2000;85 (3):1074-80. (PMID: 10720042)

13. Neely EK, Hintz RL, Wilson DM, et al. Normal ranges for immunochemiluminometric gonadotropin assays. J Pediatr 1995;127 (1):40-6. (PMID: 7608809)

14. Carretto F, Salinas-Vert I, Granada-Yvern ML, et al. The usefulness of the leuprolide stimulation test as a diagnostic method of idiopathic central precocious puberty in girls. Horm Metab Res 2014;46(13):959-63. (PMID: 25295414)

15. Kang YS, Yoo DY, Chung IH, Yoo EG. Diurnal variation of gonadotropin levels in girls with early stages of puberty. Ann Pediatr Endocrinol Metab 2017;22(3):183-8. (PMID: 29025205)

16. Pasternak Y, Friger M, Loewenthal N, Haim A, Hershkovitz E. The utility of basal serum LH in prediction of central precocious puberty in girls. Eur J Endocrinol 2012;166(2):295-9. (PMID: 22084156)

17. Mogensen SS, Aksglaede L, Mouritsen A, et al. Diagnostic work-up of 449 consecutive girls who were referred to be evaluated for precocious puberty. J Clin Endocrinol Metab 2011;96(5):1393-401. (PMID: 21346077)

18. Bangalore Krishna K, Fuqua JS, Rogol AD, et al. Use of gonadotropin-releasing hormone analogs in children: update by an international consortium. Horm Res Paediatr 2019;91(6):357-72. (PMID: 31319416)

19. Wankanit S, Mahachoklertwattana P, Pattanaprateep O, Poomthavorn P. Basal serum luteinising hormone cut-off, and its utility and cost-effectiveness for aiding the diagnosis of the onset of puberty in girls with early stages of breast development. Clin Endocrinol (Oxf) 2020;92(1):46-54. (PMID: 31705682)

20. Brito VN, Spinola-Castro AM, Kochi C, Kopacek C, Silva PC, Guerra-Júnior G. Central precocious puberty: revisiting the diagnosis and therapeutic management. Arch Endocrinol Metab 2016; 60(2):163-72. (PMID: 27191050)

21. Kumar M, Mukhopadhyay S, Dutta D. Challenges and controversies in diagnosis and management of gonadotropin dependent precocious puberty: An Indian perspective. Indian J Endocrinol Metab 2015;19(2):228-35. (PMID: 25729684)

22. Yeh SN, Ting WH, Huang CY, et al. Diagnostic evaluation of central precocious puberty in girls. Pediatr Neonatol 2021;62(2): 187-94. (PMID: 33388255)

23. Lucaccioni L, McNeilly J, Mason A, et al. The measurement of urinary gonadotropins for assessment and management of pubertal disorder. Hormones (Athens) 2016;15(3):377-84. (PMID: 27838606)

24. Bourguignon JP, Vanderschueren-Lodeweyckx M, Reuter AM, Vrindts-Gevaert Y, Gerard A, Franchimont P. Radioimmunoassays of unextracted gonadotrophins in timed fractions of 24-hour urine: morning increase of gonadotrophin excretion, a circadian pattern in relation to puberty. Horm Res 1980;13(6):367-84. (PMID: 6792026)

25. Ben Z, Ma Y, Chen C, et al. Trajectories and characteristics of urinary gonadotropin and sex hormones in healthy 0-6 years old children. Chin J Public Health 2020;36(7):1068-74. http://www.zgggws.com/en/article/doi/10.11847/zgggws1120296

26. Demir A, Voutilainen R, Juul A, et al. Increase in first morning voided urinary luteinizing hormone levels precedes the physical onset of puberty. J Clin Endocrinol Metab 1996;81(8):2963-7. (PMID: 8768859)

27. Zhang W, Xu Z, Lin S, Ma Y, Pang X, Wang Q. Correlation between nocturnal urinary gonadotropin and onset of hypothalamic-pituitary-gonadal axis in children. J Appl Clin Pediatr 2010;25(8): 561-3. http://rs.yiigle.com/CN101070201008/1364674.htm

28. Xie S, Xu Z, Ma Y, Zhang T, Wang X, Wang Q. Correlation between urinary gonadotropin and onset of hypothalamic-pituitary-gonadal axis during daytime and nighttime in children. J Appl Clin Pediatr 2011;26(20):1560-3. http://rs.yiigle.com/CN101070201120/1362928.htm

29. Zung A, Burundukov E, Ulman M, et al. The diagnostic value of first-voided urinary LH compared with GNRH-stimulated gonadotropins in differentiating slowly progressive from rapidly progressive precocious puberty in girls. Eur J Endocrinol 2014;170 (5):749-58. (PMID: 24536086)

30. Demir A, Voutilainen R, Stenman UH, Dunkel L, Albertsson-Wikland K, Norjavaara E. First Morning Voided Urinary Gonadotropin Measurements as an Alternative to the GnRH Test. Horm Res Paediatr 2016;85(5):301-8. (PMID: 27011314)

31. Yüce Ö, Bideci A, Çelik N, Çamurdan O, Cinaz P. Diagnostic value of urinary luteinizing hormone levels in the monitoring of precocious puberty treatment. Arch Endocrinol Metab 2020;64 (2):121-7. (PMID: 32236310)

32. Kolby N, Busch AS, Aksglaede L, et al. Nocturnal urinary excretion of FSH and LH in children and adolescents with normal and early puberty. J Clin Endocrinol Metab 2017;102(10):3830-8. (PMID: 28938419)

33. Lee DM, Chung IH. Morning basal luteinizing hormone, a good screening tool for diagnosing central precocious puberty. Ann Pediatr Endocrinol Metab 2019;24(1):27-33. (PMID: 30943677)

34. Ma Y, Nie S, Ben Z, et al. The dynamic trends of urinary LH and FSH assayed by ICMA during triptorelin stimulation tests in girls - a pilot study. Clin Lab 2018;64(10):1701-8. (PMID: 30336539).

35. Wang X, Xu Z, Ma Y, Zhang W, Pang X, Wang Q. Value of urinary gonadotropin determinations during gonadotropin - releasing hormone analog stimulation testing in children. J Appl Clin Pediatr 2010;25(20):1550-2,67. http://rs.yiigle.com/CN101070201020/1365496.htm

36. Ma Y, Xu Z, Zhang T, Xie S, Wang Q, Zhao J. Value of urinary gonadotropin during gonadotropin-releasing hormone analog stimulation testing in monitoring gonadotropin - releasing hormone analog therapy for children. J Appl Clin Pediatr 2012;27 (8):599-602. http://rs.yiigle.com/CN101070201208/1362261.htm