Endocrine Connections (2025) **14** e250642
https://doi.org/10.1530/EC-25-0642

Received 2 October 2025
Accepted 1 December 2025
Available online 2 December 2025
Version of Record published 12 December 2025

RESEARCH

Diagnostic accuracy of the triptorelin stimulation test for central precocious puberty in girls

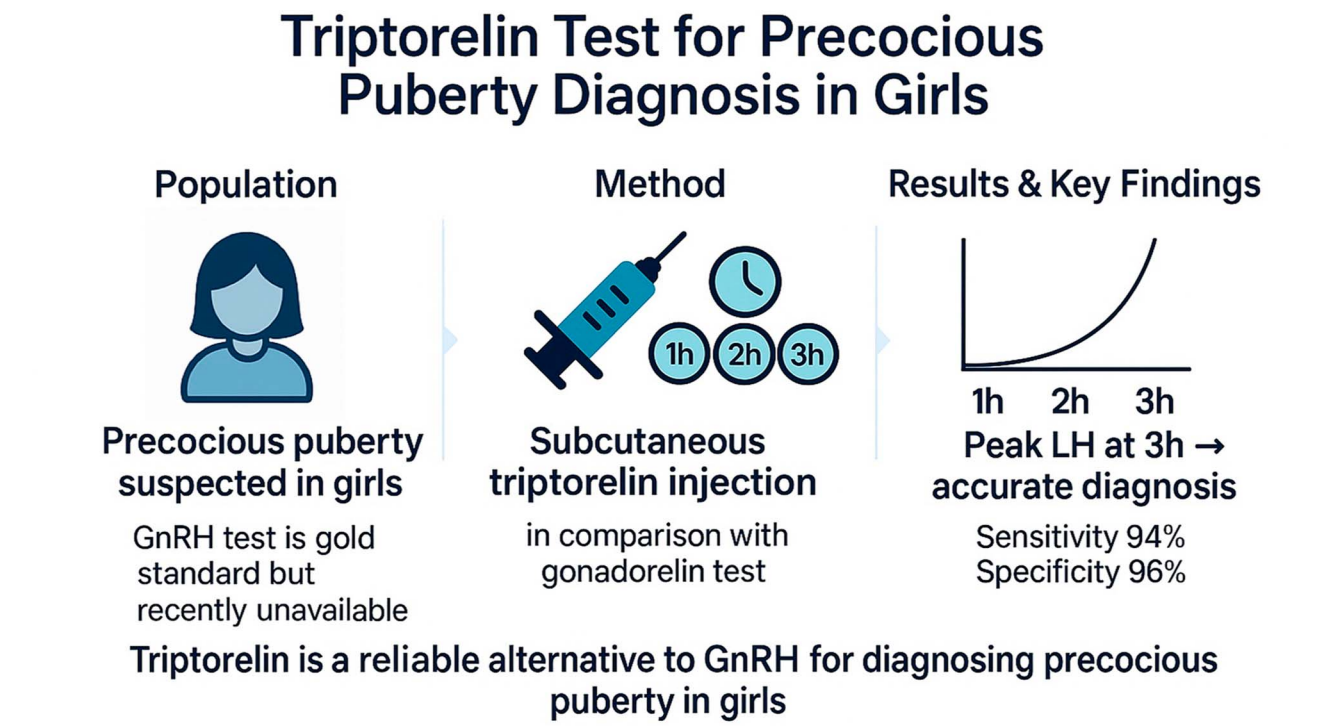
Laura Chioma[1,*], Carla Bizzarri[1,*], Marialaura Longobardi[1], Francesca Mainieri[1], Ottavia Porzio[2,3], Matteo D'Agostini[2], Laura Paone[1], Giorgia Bottaro[1] and Stefano Cianfarani[1,4,5]

[1]Endocrinology and Diabetes Unit, "Bambino Gesù" Children's Hospital, IRCCS, Rome, Italy
[2]Clinical Laboratory Unit, "Bambino Gesù" Children's Hospital, IRCCS, Rome, Italy
[3]Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italy
[4]Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy
[5]Department of Women's and Children's Health, Karolinska Institute and University Hospital, Stockholm, Sweden

Correspondence should be addressed to C Bizzarri: carla.bizzarri@opbg.net

*(L Chioma and C Bizzarri contributed equally to this work)

Graphical abstract



Published by Bioscientifica Ltd.
https://ec.bioscientifica.com

Endocrine Connections (2025) **14** e250642
https://doi.org/10.1530/EC-25-0642

Abstract

Central precocious puberty (CPP) results from premature activation of the hypothalamic–pituitary–gonadal axis. The GnRH stimulation test remains the diagnostic gold standard, especially in girls with undetectable basal LH levels. However, native GnRH is expensive and often unavailable. Rapid-acting subcutaneous triptorelin has been proposed as a reliable alternative. This retrospective study evaluated the diagnostic accuracy of subcutaneous triptorelin compared with intravenous gonadorelin and investigated the optimal timing for LH peak assessment. A total of 341 girls referred for suspected precocious puberty were evaluated: 102 underwent the triptorelin test and 239 the gonadorelin test, with gonadotropins measured by electrochemiluminescence assay at baseline and after stimulation. Based on clinical, radiological, and laboratory criteria, 143 girls were diagnosed with CPP and 198 with non-progressive thelarche (NPT). Triptorelin elicited significantly higher FSH peaks than gonadorelin, while LH peaks were comparable; consequently, FSH/LH ratios were higher after triptorelin. ROC analysis identified an optimal diagnostic LH peak cut-off of 7.14 IU/L following triptorelin administration (sensitivity 94%, specificity 96%; AUC 0.985), while a threshold of 4.7 IU/L was observed after gonadorelin (sensitivity 100%, specificity 87%; AUC 0.982). Peak LH occurred predominantly at 180 min after triptorelin in both CPP and NPT groups (78 and 90%, respectively). Despite the limitations of its retrospective and non-parallel design, this large cohort study demonstrates that subcutaneous triptorelin provides excellent diagnostic accuracy, comparable to gonadorelin. These findings support triptorelin as a reliable and accessible alternative for CPP diagnosis and contribute to the standardization of diagnostic protocols in clinical practice.

Plain language summary

This study tested triptorelin stimulation as an alternative to the standard gonadorelin test to diagnose precocious puberty in girls. Triptorelin proved equally accurate and reliable, especially when hormone levels are measured 3 h after injection, offering a practical diagnostic tool in clinical practice.

Keywords: central precocious puberty; triptorelin stimulation test; gonadorelin stimulation test; girls

Introduction

Puberty represents the transitional phase from childhood to adulthood, initiated by the reactivation of the hypothalamic–pituitary–gonadal (HPG) axis (1). Precocious puberty is defined as the premature activation of the HPG axis, resulting in the development of secondary sexual characteristics before the age of 8 years in girls and 9 years in boys (2). Precocious puberty is classified into gonadotropin-releasing hormone (GnRH)-dependent precocious puberty, also called central precocious puberty (CPP), and GnRH-independent precocious puberty, also known as peripheral precocious puberty (PPP). Over the last decades, the prevalence of CPP has been increasing worldwide, particularly among girls (2, 3).

The diagnosis of CPP relies on the presence of clinical and instrumental signs of pubertal development – such as breast enlargement in girls or increased testicular volume in boys, associated with bone age (BA) advancement and pubertal uterine morphology – together with biochemical evidence of HPG axis activation. Basal gonadotropin levels (follicle-stimulating hormone, FSH, and luteinizing hormone, LH) may support the diagnosis, particularly when basal LH is detectable (4, 5, 6, 7). However, this diagnostic approach tends to be more sensitive in boys than in girls (6). In early stages of pubertal activation in girls, clinically relevant estradiol (E2) production may occur despite low LH levels and low FSH/LH ratios. Therefore, when basal hormone levels combined with clinical and radiological findings are inconclusive, dynamic testing with GnRH stimulation remains necessary (7). The LH peak after GnRH stimulation is widely considered the gold standard for diagnosing CPP in girls (2, 8). Diagnostic cut-off values depend on the assay used; with the current ultrasensitive electrochemiluminescence methods, a peak LH concentration ≥5.0 IU/L, alone or in association with an FSH/LH ratio <1, is commonly used to confirm pubertal activation of the HPG axis in girls with premature thelarche (9, 10). On the other hand, girls presenting with early thelarche and an intermediate clinical picture, also known as 'thelarche variant' or nonprogressive precocious puberty, are characterized by mild instrumental signs of estrogen stimulation (without advanced BA or uterine maturation) and an FSH-predominant response, with a peak LH response

Endocrine Connections (2025) **14** e250642
https://doi.org/10.1530/EC-25-0642

above the threshold of 5 IU/L following the gonadorelin test (11, 12).

Nevertheless, native GnRH (gonadorelin) is expensive and, in recent years, has become unavailable in many countries due to supply issues. Conversely, GnRH agonists – widely used in reproductive medicine – have been investigated as potential alternatives for diagnostic testing in precocious puberty (13, 14). These agents act as GnRH receptor agonists, initially stimulating the release of LH and FSH upon binding with GnRH receptors. After the first phase of rapid release, continuous exposure to GnRH agonists leads to pituitary desensitization, receptor downregulation, and finally suppression of the HPG axis. The long-term safety and efficacy of depot GnRH agonists in CPP treatment are well established, and they remain the therapy of choice (1, 2, 15). Among these, triptorelin displays a longer half-life and stronger receptor affinity than native GnRH. While depot formulations are used to suppress the HPG axis in CPP, rapid-acting triptorelin acetate is employed for ovulation induction and, more recently, as a stimulus for the diagnostic evaluation of HPG axis function. After a single subcutaneous administration, serum triptorelin reaches peak levels within 15–60 min, inducing an acute stimulatory effect on gonadotropin-secreting cells, with FSH and LH reaching their maximum levels within a few hours. This stimulatory effect persists for at least 24 h due to the prolonged half-life of triptorelin, enabling additional assessment of sex-hormone secretion (16, 17).

The present study aimed to evaluate the diagnostic validity of the triptorelin stimulation test in comparison with the gonadorelin stimulation test in the diagnostic work-up of CPP. Furthermore, since triptorelin is administered subcutaneously, whereas gonadorelin (native GnRH) is given intravenously, the time to reach gonadotropin peaks may differ according to the route of administration. In line with previous studies (13, 14, 18, 19), we also aimed to determine the optimal time points for blood sampling to accurately capture the LH peak response.

Materials and methods

Subjects

We conducted an observational analysis of the medical records from girls referred for suspected precocious puberty between September 2024 and June 2025 who underwent a subcutaneous triptorelin stimulation test (Decapeptyl, Ipsen). These cases were compared with a cohort of girls evaluated between 2020 and 2022 who underwent a gonadorelin stimulation test with native GnRH (Lutrelef; Ferring). All data were collected from the outpatient clinical database of our institution.

All girls were followed for at least 3 months in order to evaluate clinical progression and complete the diagnostic work-up. Girls presenting with premature and progressive breast development, in association with at least two of the following three diagnostic criteria: clinical (increased growth velocity during follow-up), instrumental (BA advancement by more than 1 year and/or pelvic ultrasound evidence of pubertal uterine maturation), and biochemical (detectable basal LH and/or estradiol levels) parameters, were defined as CPP cases. The remaining subjects were classified as affected by non-progressive thelarche (NPT). Girls younger than 3 years who were referred for premature thelarche were excluded from the analysis.

All girls with CPP underwent magnetic resonance imaging of the hypothalamus-pituitary region to exclude intracranial pathology. Organic CPP and secondary CPP cases, due to uncontrolled PPP associated with prolonged sex steroid exposure were excluded.

Anthropometric data and medical history

Data collected included chronological age, height (H), weight (W), body mass index (BMI), pubertal stage, BA, ethnicity, family history of CPP, maternal age at menarche, and history of adoption. Height (cm) and weight (kg) were also expressed as age- and sex-specific standard deviation scores (SDS) according to Italian growth charts (20). BMI was calculated as weight divided by height squared (kg/m^2) and expressed as SDS. Pubertal stages were assessed using Tanner's criteria (21).

Laboratory measurements

Serum FSH and LH levels, both at baseline and following stimulation with gonadorelin or triptorelin, as well as basal estradiol concentrations, were determined using electrochemiluminescence immunoassay on the same technology for all measurements (Cobas e801, Roche Diagnostics, Switzerland). Estradiol intra- and interassay coefficients of variation at 27.4 pg/mL (101 pmol/L) were 6.7 and 10.6%, respectively. The analytical measuring range for estradiol assay was 5.0 pg/mL (18.4 pmol/L) (with limit of detection, LOD, corresponding to 95% confidence interval, 3 pg/mL, 11 pmol/L) to 3,000 pg/mL (11,010 pmol/L). The LH method has been standardized to the 2nd international standard NIBCS 80/552: intra- and interassay variability coefficients at 6.15 mU/mL were 1.2 and 2.0%, respectively, with an analytical measuring range from 0.3 mU/mL (with LOD 0.1 mU/mL) to 200.0 mU/mL. The FSH method has been standardized to the 2nd standard IRP 78/549: intra- and interassay variability coefficients at 1.27 mU/mL were 0.8 and 3.2%, respectively, with an analytical measuring range from 0.3 mU/mL (with LOD 0.1 mU/mL) to 200.0 mU/mL.

Endocrine Connections (2025) **14** e250642
https://doi.org/10.1530/EC-25-0642

The gonadorelin stimulation test was performed with intravenous administration of GnRH (Lutrelef; Ferring, Germany) at a dosage of 100 μg, with FSH and LH measured at baseline and 30, 60, and 90 min after the injection. The triptorelin stimulation test was conducted with the subcutaneous injection of rapid-acting triptorelin acetate (Decapeptyl; Ipsen Pharma Biotech SAS, France) at a dosage of 100 μg per m^2 to a maximum of 100 μg, with FSH and LH measured at baseline and 60, 120, and 180 min after the injection. Pharmacokinetic data suggest that after subcutaneous administration of rapid-acting triptorelin acetate, the systemic bioavailability of triptorelin is close to 100%. The elimination half-life of triptorelin is approximately 3–5 h, indicating that triptorelin is eliminated within 24 h (17).

Peak FSH and LH were defined as the maximal serum concentrations observed in response to pharmacological stimulation. The FSH/LH ratio was calculated based on the peak concentrations of FSH and LH. For the triptorelin test, LH levels at 60 min (LH-T1), 120 min (LH-T2), and 180 min (LH-T3) were analyzed.

Imaging

All subjects underwent pelvic ultrasound to evaluate uterine and ovarian morphology. A uterine longitudinal diameter above 36 mm and the presence of the endometrial echo pattern were considered indicative of estrogenic stimulation, consistent with precocious puberty.

BA was assessed in all subjects by an X-ray of the left hand and wrist according to the Greulich & Pyle method (22). BA advancement (BA−CA; years) was calculated as the difference between BA and chronological age (CA).

Statistical analysis

Data were expressed as mean ± SD for normally distributed variables and as median (interquartile range, IQR) for non-normally distributed parameters. Categorical variables were reported as number and percentage. The observed subjects were divided into two groups according to the final diagnosis (NPT and CPP). Each group was further divided into two subgroups according to the stimulation test performed (triptorelin or gonadorelin).

Categorical variables were compared using the chi-square (χ^2) test. For continuous variables, Student's *t*-test was used when normally distributed, and the Mann–Whitney U test when not normally distributed.

Spearman's correlation analysis was performed to examine the relationship between LH peak and LH values at 60, 120 and 180 min (LH-T1, LH-T2, and LH-T3, respectively) after triptorelin administration.

In order to evaluate the ability of peak LH values following gonadorelin and triptorelin stimulation to discriminate CPP from NPT, receiver operating characteristic (ROC) curve analysis was performed. The area under the curve (AUC) was used to evaluate diagnostic performance. The Youden index was applied in order to estimate the optimal cut-off with the best performance in terms of both sensitivity and specificity.

The Youden index (J) is a function of Se (c) and Sp (c), such that $J(c) = \{Se(c) + Sp(c) - 1\} = \{Se(c) - (1 - Sp(c))\}$ over all cut-points c; the optimal cutpoint is the one for which the value J is maximum.

All statistical analyses were performed with the statistical package SPSS v23 for Windows (SPSS Inc, USA), and a P-value of $P < 0.05$ was considered statistically significant.

Ethics approval

Clinical, laboratory, and radiological data of patients were obtained and processed after signed informed consent from parents/guardians. The study was conducted in accordance with the Declaration of Helsinki and its subsequent amendments to ensure data confidentiality and protection. No further approval from an external Ethics Committee was required. The Independent Institutional Review Board approved the study.

Results

The study population consisted of 341 girls, of whom 239 were observed between 2020 and 2022 and underwent the gonadorelin stimulation test (93 with CPP and 146 with NPT), and 102 were observed between September 2024 and June 2025 and underwent the triptorelin stimulation test (50 with CPP and 52 with NPT) (Fig. 1). The study population was further divided into two diagnostic groups: 143 girls with CPP and 198 girls with

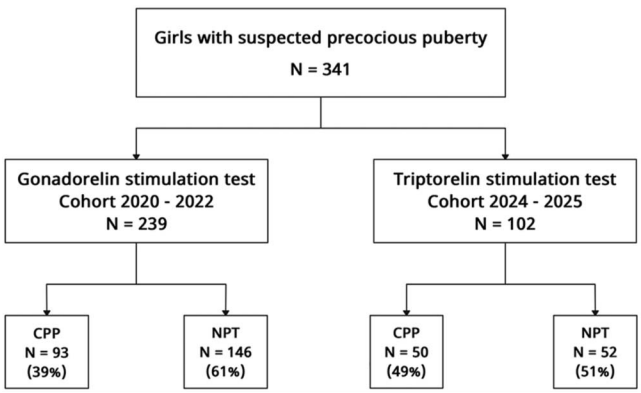


Figure 1

Flowchart summarizing the study design.

Table 1 Anthropometric characteristics and instrumental parameters of the study population.

	CPP (*n* = 143)			NPT (*n* = 198)		
	Gonadorelin	**Triptorelin**	***P***	**Gonadorelin**	**Triptorelin**	***P***
Number (%)	93 (65)	50 (35)		146 (74)	52 (26)	0.08
Age (years)	7.65 ± 0.58	7.78 ± 0.56	0.61	7.19 ± 0.79	7.33 ± 0.82	0.28
Height SDS	0.91 ± 1.06	1.01 ± 1.07	0.97	0.53 ± 0.95	0.67 ± 1.17	0.40
Weight SDS	0.56 ± 0.88	0.41 ± 1.09	0.37	0.44 ± 1.05	0.43 ± 1.14	0.98
BMI SDS	0.32 ± 0.91	−0.01 ± 1.21	0.17	0.31 ± 1.10	0.22 ± 1.24	0.61
BMI ≥ 1 SDS *n* (%)	23 (25)	12 (24)	0.92	41 (28)	14 (27)	0.87
BMI ≥ 2 SDS *n* (%)	1	1	NA	8	6	NA
BSA (m^2)	1.05 ± 0.14	1.04 ± 0.14	0.68	0.99 ± 0.15	1.01 ± 0.18	0.44
BA−CA	1.83 ± 1.12	1.67 ± 0.86	0.07	0.89 ± 0.86	0.63 ± 0.96	0.08
Tanner stage (breast), number (%)	II: 65 (70) III: 28 (30)	II: 40 (80) III: 10 (20)	0.19	II: 143 (98) III: 3 (2)	II: 51 (98) III: 1 (2)	0.95
Uterine longitudinal diameter (mm)	41.58 ± 8.52	40.35 ± 6.92	0.21	32.68 ± 7.99	32.07 ± 5.66	0.62

CPP, central precocious puberty; NPT, non-progressive precocious thelarche; SDS, standard deviation score; BMI, body mass index; BSA, body surface area; BA, bone age; CA, chronological age; NA, not available. Parameters are expressed as mean ± SD if not differently indicated. Student's *t*-test was applied to compare variables with normal distribution. Categorical variables were compared using chi-square (χ^2) test.

NPT. In the CPP group, 93 subjects (65%) underwent the gonadorelin stimulation test, while 50 subjects (35%) received the triptorelin stimulation test. In the NPT group, 146 subjects (74%) were tested with gonadorelin, and 52 subjects (26%) with triptorelin. Anthropometric and instrumental data for both CPP and NPT groups, further subdivided according to the type of stimulation test performed (triptorelin or gonadorelin), are presented in Table 1. Within the two diagnostic groups (CPP or NPT), no significant differences in anthropometric characteristics or pubertal stage were observed between triptorelin and gonadorelin subgroups.

Among subjects of the CPP group, we observed that 20 out of 93 (22%) girls tested with gonadorelin and 17 out of 50 (34%) of those investigated with triptorelin had undetectable baseline LH levels (P = 0.10); while 60 out of 93 (65%) girls in the gonadorelin subgroup and 32 out of 50 (64%) girls in the triptorelin subgroup had detectable baseline estradiol (P = 0.95).

The triptorelin test elicited significantly higher peak FSH levels compared with the gonadorelin test in both CPP (16.40 (7.55) IU/L vs 11.70 (4.98) IU/L, P = 0.0001) and NPT groups (16.60 (9.70) IU/L vs 11.05 (4.68) IU/L, P = 0.0001) (Fig. 2A). By contrast, no significant differences in peak LH levels were observed between the triptorelin and gonadorelin subgroups: 14.00 (21.55) IU/L vs 18.6 (19.50) IU/L (P = 0.67) in CPP group; 3.49 (2.51) IU/L vs 3.09 (1.89) IU/L (P = 0.09) in the NPT group (Table 2, Fig. 2B).

Consequently, the FSH/LH ratio was significantly higher in the triptorelin subgroups compared with the gonadorelin subgroups: 0.98 (1.05) vs 0.63 (0.55) (P = 0.0001) in the CPP group and 5.13 (2.05) vs 3.51 (1.99) (P = 0.0001) in the NPT group (Table 2, Fig. 2C).

In the NPT group, the LH peak occurred predominantly at LH-T3 (47/52 subjects, 90%), whereas only two and three subjects showed a peak at LH-T1 and LH-T2, respectively (χ^2 goodness-of-fit test, P < 0.0001). Spearman's correlation analysis confirmed the strongest association between LH peak and LH-T3 (r = 0.995, P < 0.0001), compared to LH-T1 (r = 0.901) and LH-T2 (r = 0.961) (Fig. 3A). Similarly, in the CPP group, the LH peak was most frequently observed at LH-T3 (39/50 subjects, 78%), compared with seven subjects at LH-T1 and four at LH-T2 (χ^2 goodness-of-fit test, P < 0.0001). Spearman's correlation analysis confirmed the strongest relationship between LH peak and LH-T3 (r = 0.993, P < 0.0001) compared to LH-T1 (r = 0.915) and LH-T2 (r = 0.968) (Fig. 3B).

No significant differences in basal FSH, LH, and estradiol levels were observed between the triptorelin and gonadorelin subgroups within either diagnostic group (Table 2).

ROC analysis with the Youden index function was performed to estimate the best cut-off of peak LH in order to distinguish CPP from NPT subjects. After triptorelin stimulation, the optimal cut-off was 7.14 IU/L, yielding a sensitivity of 94% and a specificity of 96% (AUC 0.985; 95% CI: 0.967–1.00) (Table 3, Fig. 4A). After gonadorelin stimulation, the optimal cut-off was 4.70 IU/L, with a sensitivity of 100% and a specificity of 87% (AUC 0.982; 95% CI: 0.969–0.995) (Table 3, Fig. 4B).

Discussion

This study represents the largest cohort of girls with CPP assessed using the triptorelin stimulation test to date. An optimal diagnostic LH peak cut-off of 7.14 IU/L was identified following triptorelin administration.

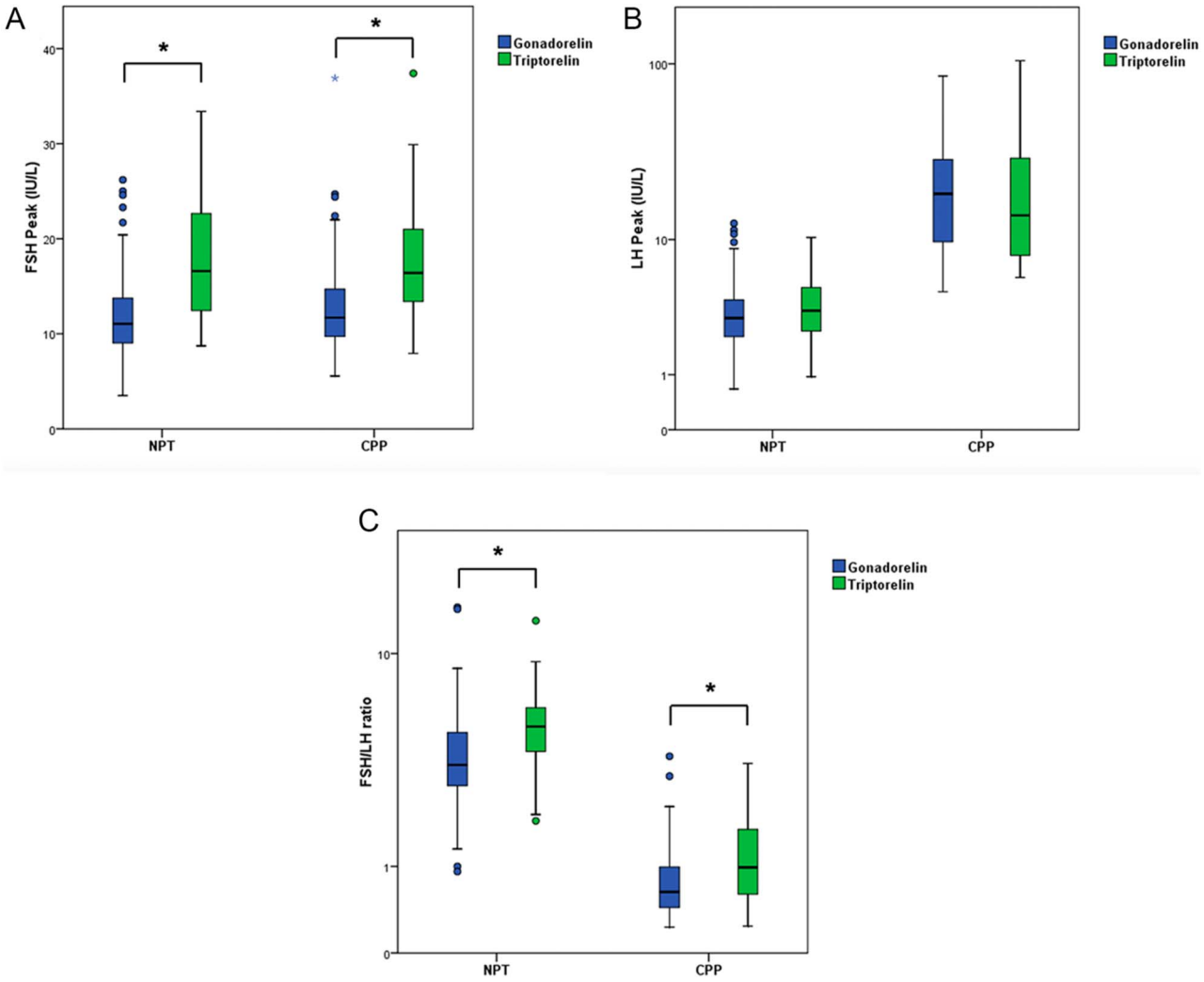


Figure 2

Comparison of FSH peak, LH peak, and FSH/LH ratio after gonadorelin and triptorelin stimulation test between NPT and CPP. (A) Boxplot representing median, IQR, and outliers of FSH peak values between the two groups. (B) Boxplot representing median, IQR, and outliers of peak LH values between the two groups. (C) Boxplot representing median, IQR, and outliers of FSH/LH ratio values between the two groups. Mann–Whitney U test was applied, *P < 0.001.

This finding is consistent with previously published data; however, considerable variability in the proposed thresholds has been reported in the literature, likely reflecting differences in patient selection, stimulation protocols, assay methodologies, and the limited sample sizes of individual studies. Early retrospective evidence suggested a diagnostic threshold of 6 IU/L, with samples collected at baseline and at 30, 60, 90, and 120 min after

Table 2 Laboratory parameters of the study population.

	CPP			NPT		
	Gonadorelin	**Triptorelin**	***P***	**Gonadorelin**	**Triptorelin**	***P***
Basal FSH (IU/L)	3.70 (2.43)	3.45 (3.15)	0.57	1.60 (1.20)	1.75 (1.10)	0.95
Basal LH (IU/L)	0.90 (1.63)	0.60 (1.58)	0.10	0.00 (0)	0.00 (0)	0.27
FSH peak (IU/L)	11.70 (4.98)	16.40 (7.55)	<0.0001	11.05 (4.68)	16.60 (9.7)	<0.0001
LH peak (IU/L)	18.60 (19.50)	14.00 (21.55)	0.67	3.09 (1.89)	3.49 (2.51)	0.09
FSH/LH ratio	0.63 (0.55)	0.98 (1.05)	<0.0001	3.51 (1.99)	5.13 (2.05)	<0.0001
17-beta-estradiol (pg/mL)	16.75 (32.03)	10.60 (32.25)	0.82	0.00 (0)	0.00 (0)	0.79

CPP, central precocious puberty; NPT, non-progressive precocious thelarche. Not-normally distributed parameters are expressed as median (interquartile range or IQR) and compared using the Mann–Whitney U test.

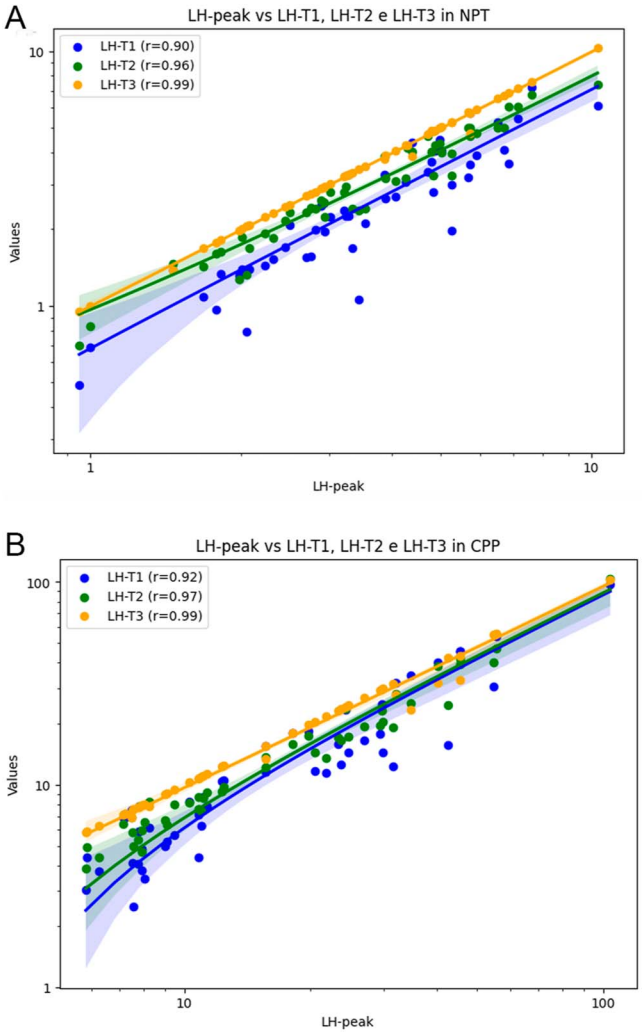


Figure 3

(A) Spearman's correlation between LH peak and LH values at 60, 120, and 180 min (LH-T1, LH-T2, and LH-T3, respectively) after triptorelin test in the NPT group. (B) Spearman's correlation between LH peak and LH-T1, LH-T2, and LH-T3 after triptorelin test in the CPP group.

triptorelin administration (13). A randomized controlled trial subsequently compared the intravenous GnRH test (100 μg/m^2) with the subcutaneous triptorelin test (0.1 mg/m^2 of rapid-acting triptorelin to a maximum of 0.1 mg) in 46 girls with suspected CPP. Gonadotropin levels were measured at 0, 3, and 24 h after the administration. This study reported that an LH response measured at 3 h after the administration of rapid-acting triptorelin >7 IU/L by immunofluorometric assay (IFMA) or >8 IU/L by electrochemiluminescence immunoassay (ECLIA) confirmed the diagnosis of CPP with 100% specificity and 76% sensitivity (14). The same study also highlighted the usefulness of estradiol measurement at 24 h post-stimulus in order to identify pubertal evolution in patients with borderline LH response. A prospective study proposed a lower diagnostic threshold, identifying an LH peak of 3.4 IU/L measured 180 min after subcutaneous triptorelin administration for the diagnosis of CPP (18). On the contrary, a Korean cohort study reported a cut-off of peak LH ≥ 4.5 IU/L at 120 min after triptorelin injection as indicative of CPP (19). More recently, an Italian group questioned the historical use – adopted at their own institution – of a high LH peak threshold (15 IU/L) for the diagnosis of CPP, demonstrating that many girls with LH peaks below this level nonetheless exhibited clear pubertal progression. The triptorelin stimulation test was repeated when clinical signs of pubertal advancement, such as progression in Tanner stage and increased growth velocity, were observed. These patients had significantly higher LH peaks at the first evaluation (LH peak >5 UI/L in 55%), with higher basal LH levels and elevated LH/FSH ratios. Based on their findings, they proposed a new diagnostic LH peak threshold of 5 IU/L (23).

Taken together, these findings underscore the lack of standardization in the interpretation of the triptorelin test. In our study, we deliberately avoided defining the diagnosis of CPP solely on the basis of the LH peak value. This methodological choice strengthens the reliability of the ROC analysis, allowing for a more accurate determination of the optimal cut-off to discriminate CPP from non-progressive forms.

The ROC analysis of the gonadorelin test confirmed the diagnostic accuracy of this test, already extensively described in the literature, identifying an ideal cut-off of 4.70 IU/L in line with previous data (1, 2, 15). From our perspective, considering the specific differences in sampling times, diagnostic threshold of LH peak, and test duration, the two tests may be used interchangeably, as they exhibit comparable diagnostic accuracy.

In both CPP and NPT cohorts, the LH peak was observed 180 min after triptorelin administration in the vast majority of cases, as confirmed by the best correlation

Table 3 ROC analysis. The ideal cut-off of peak LH in order to distinguish CPP from NPT with triptorelin and gonadorelin stimulation tests.

	Ideal cut-off	AUC	CI	*J*	Sensitivity	Specificity
Triptorelin peak LH (IU/L)	7.14	0.985	0.967–1.000	0.902	94%	96%
Gonadorelin peak LH (IU/L)	4.70	0.982	0.969–0.995	0.871	100%	85%

CPP, central precocious puberty; NPT, non-progressive precocious thelarche; AUC, area under the curve; CI, confidence interval; J, Youden index.

Endocrine Connections (2025) **14** e250642
https://doi.org/10.1530/EC-25-0642

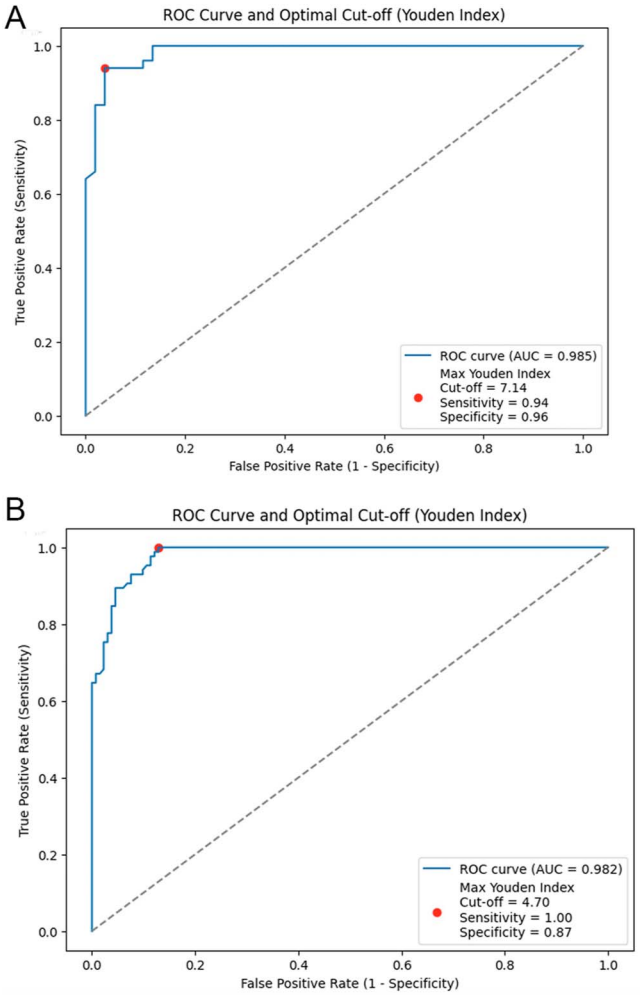


Figure 4

(A) ROC curve analysis with ideal cut-off of LH peak after triptorelin in order to distinguish the CPP from NPT subjects. (B) ROC curve analysis with ideal cut-off of LH peak after gonadorelin in order to distinguish the CPP from NPT subjects.

between LH peak values and LH levels measured at 180 min. This is consistent with the slower absorption of triptorelin from subcutaneous tissue. Nevertheless, the proportion of CPP subjects with earlier peaks (at 60 or 120 min) was higher than in the NPT group (22 versus 10%), suggesting greater variability in LH dynamics during early pubertal activation. Evidence from the literature regarding the timing of the LH peak after triptorelin is less robust. While most studies report the LH peak at 180 min (14, 18), some have observed peaks at 60 or 120 min. However, these latter observations are based on studies with small sample sizes (24), limited monitoring durations up to 120 min (13), or incomplete assessment at 180 min (19). Based on our results, a simplified single-sample approach at 180 min may represent a feasible, cost-effective, and less invasive alternative to traditional multi-sample GnRH stimulation protocols. This strategy could enhance the practicality of CPP diagnostic testing, particularly in low-resource or high-throughput clinical settings. Further prospective, standardized studies are warranted to confirm these findings and to strengthen the evidence supporting this streamlined diagnostic approach for CPP evaluation.

We also found that triptorelin elicited a significantly higher FSH peak compared to gonadorelin, in keeping with its stronger pharmacological activity. Although LH peaks were slightly higher after triptorelin than after gonadorelin, the difference was not statistically significant. As a consequence, the FSH/LH ratio was significantly higher in the triptorelin subgroups, both in CPP and NPT. Importantly, in a considerable proportion (25/50, 50%) of girls with clinically confirmed CPP undergoing the triptorelin test, the FSH/LH ratio exceeded 1, thereby limiting the utility of this parameter in the definition of CPP.

Our data confirm that the measurement of basal gonadotropin levels alone may be insufficient to establish or exclude the diagnosis of CPP in girls, underlying the previously reported importance of the LH value after stimulation in the diagnostic work-up (7). In fact, we observed that 20 out of 93 (22%) girls tested with gonadorelin and 17 out of 50 (34%) of those investigated with triptorelin had undetectable basal LH levels despite a confirmed clinical diagnosis of CPP and the use of an ultrasensitive LH measurement method.

We acknowledge that the design of our study may represent a limitation. In particular, the study population did not undergo both stimulation tests, which would have allowed for a more direct comparison between the triptorelin and gonadorelin tests. To mitigate this limitation, the CPP and NPT subgroups assessed with the two different tests were homogeneous with respect to anthropometric and pubertal characteristics, imaging findings, and basal levels of FSH, LH, and estradiol.

Due to logistical constraints, we were unable to prolong the test duration beyond 180 min or measure estradiol levels at 24 h, which, as noted by some authors, could have further enhanced diagnostic accuracy (14). On the other hand, the measurement of estradiol at 24 h after triptorelin administration would likely not have significantly improved diagnostic accuracy for CPP, given the basal estradiol levels already detectable in the majority (72%) of CPP subjects tested with triptorelin.

In conclusion, our findings support the use of the subcutaneous triptorelin test as a reliable diagnostic tool for CPP. The diagnostic LH peak threshold of 7.14 IU/L provided excellent sensitivity and specificity in the largest cohort of girls with CPP investigated with triptorelin to date. The test appears to be particularly useful in cases with suggestive clinical features but undetectable baseline LH levels, where stimulation is

essential to confirm the diagnosis. Although variability in proposed thresholds persists across studies, our results contribute to the effort toward harmonizing diagnostic criteria and highlight the importance of standardized protocols in clinical practice.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the work reported.

Funding

This work was supported by the Italian Ministry of Health with current research funds.

Author contribution statement

LC, CB, and SC contributed to the study conception and design. Material preparation, data collection, and analysis were performed by ML, FR, LP, and GB. The first draft of the manuscript was written by LC and CB. All authors reviewed the draft and approved the final manuscript.

References

1 Zevin EL & Eugster EA. Central precocious puberty: a review of diagnosis, treatment, and outcomes. *Lancet Child Adolesc Health* 2023 **7** 886–896. (https://doi.org/10.1016/s2352-4642(23)00237-7)

2 Carel JC & Léger J. Clinical practice. Precocious puberty. *N Engl J Med* 2008 **358** 2366–2377. (https://doi.org/10.1056/NEJMcp0800459)

3 Parent AS, Teilmann G, Juul A, *et al.* The timing of normal puberty and the age limits of sexual precocity: variations around the world, secular trends, and changes after migration. *Endocr Rev* 2003 **24** 668–693. (https://doi.org/10.1210/er.2002-0019)

4 Pasternak Y, Friger M, Loewenthal N, *et al.* The utility of basal serum LH in prediction of central precocious puberty in girls. *Eur J Endocrinol* 2012 **166** 295–299. (https://doi.org/10.1530/eje-11-0720)

5 Lee HS, Park HK, Ko JH, *et al.* Utility of Basal luteinizing hormone levels for detecting central precocious puberty in girls. *Horm Metab Res* 2012 **44** 851–854. (https://doi.org/10.1055/s-0032-1321905)

6 Yazdani P, Lin Y, Raman V, *et al.* A single sample GnRHa stimulation test in the diagnosis of precocious puberty. *Int J Pediatr Endocrinol* 2012 **2012** 23. (https://doi.org/10.1186/1687-9856-2012-23)

7 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 Endocrinolology Metab* 2011 **96** 1393–1401. (https://doi.org/10.1210/jc.2010-2745)

8 Resende EA, Lara BH, Reis JD, *et al.* Assessment of basal and gonadotropin-releasing hormone-stimulated gonadotropins by immunochemiluminometric and immunofluorometric assays in normal children. *J Clin Endocrinol Metab* 2007 **92** 1424–1429. (https://doi.org/10.1210/jc.2006-1569)

9 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** 357–372. (https://doi.org/10.1159/000501336)

10 Neely EK, Hintz RL, Wilson DM, *et al.* Normal ranges for immunochemiluminometric gonadotropin assays. *J Pediatr* 1995 **127** 40–46. (https://doi.org/10.1016/s0022-3476(95)70254-7)

11 Burlo F, Lorenzon B, Tamaro G, *et al.* Prevalence and characteristics of thelarche variant. *Front Endocrinol* 2023 **14** 1303989. (https://doi.org/10.3389/fendo.2023.1303989)

12 Stanhope R & Brook CC. Thelarche variant: a new syndrome of precocious sexual maturation? *Acta Endocrinol* 1990 **123** 481–486. (https://doi.org/10.1530/acta.0.1230481)

13 Poomthavorn P, Khlairit P & Mahachoklertwattana P. Subcutaneous gonadotropin-releasing hormone agonist (triptorelin) test for diagnosing precocious puberty. *Horm Res Paediatr* 2009 **72** 114–119. (https://doi.org/10.1159/000232164)

14 Freire AV, Escobar ME, Gryngarten MG, *et al.* High diagnostic accuracy of subcutaneous triptorelin test compared with GnRH test for diagnosing central precocious puberty in girls. *Clin Endocrinol* 2013 **78** 398–404. (https://doi.org/10.1111/j.1365-2265.2012.04517.x)

15 Eugster EA. Treatment of central precocious puberty. *J Endocr Soc* 2019 **3** 965–972. (https://doi.org/10.1210/js.2019-00036)

16 Mason-Garcia M, Vigh S, Comaru-Schally AM, *et al.* Radioimmunoassay for 6-D-tryptophan analog of luteinizing hormone-releasing hormone: measurement of serum levels after administration of long-acting microcapsule formulations. *Proc Natl Acad Sci U S A* 1985 **82** 1547–1551. (https://doi.org/10.1073/pnas.82.5.1547)

17 Lahlou N, Carel JC, Chaussain JL, *et al.* Pharmacokinetics and pharmacodynamics of GnRH agonists: clinical implications in pediatrics. *J Pediatr Endocrinol Metab* 2000 **13** 723–737. (https://doi.org/10.1515/jpem.2000.13.s1.723)

18 Vukovic R, Milenkovic T, Soldatovic I, *et al.* Triptorelin stimulated luteinizing hormone concentrations for diagnosing central precocious puberty: study of diagnostic accuracy. *Endocrine* 2022 **75** 934–941. (https://doi.org/10.1007/s12020-021-02947-z)

19 Ahn J, Lee Y, Gim S, *et al.* Use of the subcutaneous triptorelin stimulation test for diagnosis of central precocious puberty. *Children* 2023 **10** 1830. (https://doi.org/10.3390/children10111830)

20 Cacciari E, Milani S, Balsamo A, *et al.* Italian cross-sectional growth charts for height, weight and BMI (2 to 20 year). *J Endocrinol Investig* 2006 **29** 581–593. (https://doi.org/10.1007/bf03344156)

21 Tanner JM & Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 1976 **51** 170–179. (https://doi.org/10.1136/adc.51.3.170)

22 Greulich WW & Pyle SI. *Radiographic Atlas of Skeletal Development of the Hand and Wrist*, edn 2. Stanford, CA, USA: Stanford University Press, 1959.

23 Cavarzere P, Sandri M, Arrigoni M, *et al.* Revising LH cut-off for the diagnosis of central precocious puberty via triptorelin stimulation assay. *Endocrine* 2025 **87** 842–849. (https://doi.org/10.1007/s12020-024-04055-0)

24 Kim YJ, Hwangbo J, Park KH, *et al.* Effectiveness of the triptorelin stimulation test compared with the classic gonadotropin-releasing hormone stimulation test in diagnosing central precocious puberty in girls. *Ann Pediatr Endocrinol Metab* 2024 **29** 90–94. (https://doi.org/10.6065/apem.2346054.027)