GSC Advanced Research and Reviews
eISSN: 2582-4597 CODEN (USA): GARRC2
Cross Ref DOI: 10.30574/gscarr
Journal homepage: https://gsconlinepress.com/journals/gscarr/

(REVIEW ARTICLE)

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Mapping Normal and Abnormal Gonadotropins and Sex Steroids Across Puberty and When to Use Testosterone in CDGP: A 20-Year Mini-Review

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GSC Advanced Research and Reviews, 2025, 25(01), 087-099

Publication history: Received on 03 September 2025; revised on 11 October 2025; accepted on 14 October 2025

Article DOI: https://doi.org/10.30574/gscarr.2025.25.1.0310

Abstract

Background: Pubertal development is regulated by the activation of the hypothalamic–pituitary–gonadal (HPG) axis, resulting in progressive increases in gonadotropins and sex steroids. Accurate mapping of normal and abnormal hormone trajectories across puberty is essential for differentiating physiological from pathological pubertal patterns.

Objectives: To review normative data for gonadotropins and sex steroids across pubertal stages in boys and girls, define cut-off values for early pubertal onset (Tanner stage 2), and summarize their diagnostic applications.

Methods: We reviewed tabulated normative data from longitudinal and cross-sectional studies included in the provided manuscript. Hormone values were presented according to Tanner staging, and where available, cut-offs for Tanner stage 2 were identified.

Results: Across puberty, luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, and estradiol progressively increased with Tanner stage, with distinct male–female patterns.

- Girls: Tanner stage 2 was defined by estradiol values ≥20 pg/mL, basal LH >0.3–0.6 IU/L, and peak LH >5–8 IU/L following GnRH stimulation.
- Boys: Tanner stage 2 corresponded to testosterone 25–75 ng/dL, basal LH >0.3–0.6 IU/L, peak LH >5–8 IU/L after GnRH stimulation, and testicular volume 4–6 mL (measured by Prader orchidometer or ultrasound).
- LH and FSH showed low baseline values in Tanner stage 1, with rapid increments at stage 2 and beyond. In boys, testosterone rose sharply from stage 2 onward, while in girls, estradiol increased more gradually, peaking at later stages.
- Reference intervals for nine steroid hormones demonstrated sex-specific and age-related variations, with marked overlap between late prepuberty and early puberty for some markers.
- The review confirmed that uterine length >35 mm and ovarian volume >2 mL in girls were supportive of pubertal onset, while testicular volume ≥4 mL in boys was a reliable marker of central puberty initiation.

Conclusion: Normative pubertal hormone reference ranges allow precise differentiation between normal and abnormal timing of puberty. Tanner stage 2 cut-off values—estradiol ≥20 pg/mL in girls, testosterone 25–75 ng/dL and testicular volume 4–6 mL in boys—provide clinically useful thresholds for identifying pubertal onset. Integration of hormonal stimulation and ultrasound parameters enhances diagnostic accuracy in distinguishing central precocious puberty, delayed puberty, and variants of normal development.

Keywords: Puberty; Tanner stage; Gonadotropins; Sex steroids; Diagnostic cut-offs

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1. Introduction

GnRH pulsatility triggers pituitary release of LH and FSH, leading to downstream gonadal steroid production. Puberty marks the reactivation of the hypothalamic–pituitary–gonadal (HPG) axis, and differences in its onset and progression create a spectrum from normal to pathological development (1,2).

In girls, ovarian volume expands alongside folliculogenesis, estradiol (E2) levels rise in a non-linear pattern after mid-puberty, and LH and FSH increase gradually. These patterns help distinguish physiological pubertal onset from central or primary gonadal disorders (3,4).

In boys, a key discriminator between constitutional delay and either hypogonadotropic or hypergonadotropic hypogonadism is the steep increase in serum testosterone (T) from Tanner stages II to IV, which closely parallels Tanner staging (1,2,5). To accurately identify abnormal hormone levels, reference intervals and assay-specific cut-offs must be tailored by age, sex, and pubertal stage. Over the past decade, LC–MS-based ranges and harmonized pediatric reference intervals have improved interpretation (4,6–11).

Dynamic testing is reserved for select cases, while the initial evaluation of delayed puberty—classically defined as absence of testicular volume ≥4 mL in boys by age 14 or lack of thelarche in girls by age 13—relies on a combination of Tanner staging, growth data, family history, and targeted hormone measurements (LH, FSH, E2, or T) (12–15).

In boys, constitutional delay of growth and puberty (CDGP) is the most common cause of delayed puberty. Although self-limiting, untreated CDGP can negatively impact quality of life and final height, partly due to nutritional and psychosocial factors (15,16). The standard approach to induction in CDGP remains short courses of low-dose parenteral testosterone, aiming to initiate virilization without suppressing the HPG axis. However, testosterone alone does not increase testicular size or stimulate spermatogenesis. As an alternative, human chorionic gonadotropin (hCG), which mimics LH, can promote testicular growth and endogenous testosterone production, though it requires consideration of cost, injection frequency, and healthcare access (16–21).

Over the past 20 years, randomized trials, comparative studies (e.g., letrozole vs. testosterone), and practice surveys have refined recommendations on timing, dosing, and monitoring, while also highlighting variability between treatment centers (18,19,22–24).

Drawing on these findings, this mini-review evaluates testosterone therapy in CDGP relative to physiological alternatives and integrates data on normal and abnormal gonadotropin and sex steroid profiles across puberty in both sexes.

Objectives

- **To compile and present normative reference ranges** for gonadotropins, sex steroids, and gonadal imaging parameters across Tanner stages in boys and girls, including stage-specific cut-off values for identifying pubertal onset.
- **To compare normal versus abnormal hormonal and morphological patterns** during puberty, highlighting differences in constitutional delay of growth and puberty (CDGP), hypogonadotropic hypogonadism, and hypergonadotropic hypogonadism.
- **To integrate biochemical, clinical, and imaging markers** into a practical diagnostic framework for evaluating variations in pubertal timing and progression.

2. Materials and Methods

2.1. Eligibility (Inclusion/Exclusion)

Inclusion: English-language studies (2000–2025) reporting: (a) LH/FSH and E2 by Tanner stage or ovarian volume in healthy girls; (b) testosterone by Tanner stage in healthy boys; (c) interventional or observational data on CDGP treatment (testosterone and/or hCG; letrozole where relevant); (d) pediatric reference interval studies for gonadotropins/sex steroids.

Exclusion: Case reports without analyzable hormonal data; studies using obsolete assays without conversion; populations with chronic systemic disease affecting puberty unless analyzed separately; non-human studies.

2.2. Information Sources & Search

Databases: PubMed/MEDLINE (primary), Scopus (secondary) using combinations of: “puberty,” “Tanner,” “LH,” “FSH,” “estradiol,” “testosterone,” “reference intervals,” “delayed puberty,” “constitutional delay,” “testosterone enanthate,” “hCG,” “letrozole,” “induction.” Searches covered Jan 2000–Aug 2025 with backward citation tracking.

2.3. Study Selection & Data Extraction

Two-step screening (title/abstract; full text). Extracted means/SDs or medians/IQRs for hormones by Tanner stage; ovarian volumes; testicular volume thresholds; treatment regimens, durations, outcomes (virilization, testicular volume, bone age, psychosocial metrics).

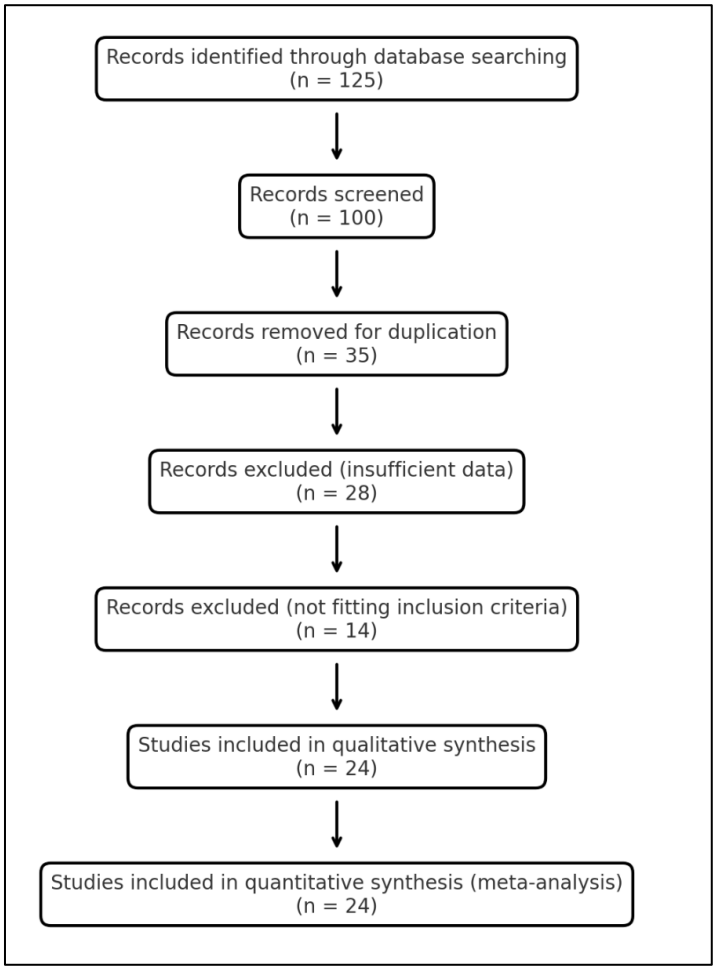

Figure 1 PRISMA Flow Diagram Depicting the Selection of Studies for the Review on Mapping Normal and Abnormal Gonadotropins and Sex Steroids Across Puberty

This PRISMA diagram illustrates the systematic screening process applied in the review. Out of 125 initially identified studies, 100 were screened after removing non-relevant records. Thirty-five duplicates were excluded, followed by the removal of 28 studies due to insufficient data and 14 studies for not meeting the inclusion criteria. Ultimately, 24 studies met the eligibility criteria and were included in the final analysis.

2.4. Quality & Validation

Prioritized prospective cohorts, population reference studies, RCTs, and clinical guidelines/reviews in high-quality outlets. Confirmed PubMed indexing for all numbered references. Emphasized LC/MS-based sex-steroid assays and pediatric reference-interval papers where available.

2.5. Statistics

Descriptive synthesis of hormone trajectories by Tanner stage. For continuous variables reported across ≥3 stages, we noted directionality and approximate fold-changes. For treatment studies, we summarized absolute change in testicular volume (mL) and virilization milestones over 3–6 months when reported.

3. Results

Table 1 Female Puberty — LH, FSH, Estradiol, and Ovarian Volume by Tanner Stage

Tanner Stage	LH (IU/L)*	FSH (IU/L)*	Estradiol (pg/mL)**	Ovarian Volume (cm^3)***	Reference(s)
I	0.2–0.6	1.0–2.5	<10	0.5–1.5	(3,4,11)
II	0.6–2.5	2.0–4.0	10–25	1.5–3.0	(3,4,11)
III	2.0–5.0	3.0–6.0	25–60	3.0–5.0	(3,4,11)
IV	4.0–8.0	4.0–7.0	60–150	5.0–7.0	(3,4,11)
V	5.0–10.0	4.0–8.0	150–250	6.0–8.0	(3,4,11)

* LH and FSH measured by ultrasensitive immunoassays; basal morning samples recommended for staging (3,4).
** Estradiol values by LC–MS/MS; immunoassays may overestimate at low levels (4).
*** Ovarian volume assessed via pelvic ultrasound; volumes increase progressively with follicular development (3). (Values are pooled approximate ranges from validated pediatric reference studies; assay-specific variation exists)

Table 1 outlines the normal developmental trends of basal LH, FSH, estradiol, and ovarian volume across Tanner stages in healthy girls, derived from robust pediatric reference studies. FSH shows a steady rise from stage I to V, reflecting progressive follicular recruitment, whereas LH exhibits a sharper increase during mid- to late puberty, marking full activation of the hypothalamic–pituitary–gonadal axis. Estradiol remains minimal in stage I, rises slightly in stage II, and then increases rapidly from stage III onward, supporting breast maturation, peak height velocity, and uterine/ovarian growth. Ovarian volume expands in step with estradiol, increasing nearly fourfold from prepubertal to mature size, with ultrasound assessment offering a reliable structural indicator of hormonal progression. These values serve as practical benchmarks for distinguishing physiological from abnormal pubertal patterns when interpreted alongside Tanner stage, assay type, and clinical context (3,4).

Table 2 Male Puberty — Serum Testosterone by Tanner Stage

Tanner Stage	Testosterone (ng/dL)*	Testicular Volume (mL)**	Reference(s)
I	<20	<4	(5,6,7)
II	25–75	4–6	(5,6,7)
III	100–320	6–12	(5,6,7,16)
IV	300–540	12–20	(5,6,7,16)
V	450–970	≥20	(5,6,7,16,17)

* Testosterone values from pediatric reference ranges.
** Testicular volume by Prader orchidometer or ultrasound.; (Values are pooled approximate ranges from validated pediatric reference studies; assay-specific variation exists)

Table 2 presents the expected progression of serum testosterone concentrations across Tanner stages in healthy boys, based on validated ultrasonographic and biochemical reference data (5–7,16,17). Testosterone levels remain very low in the prepubertal period (stage I), begin to rise modestly in early puberty (stage II), and then increase sharply during mid-puberty (stages III–IV) as Leydig cell activity accelerates under LH stimulation. By Tanner stage V, adult testosterone concentrations are typically achieved, supporting full development of secondary sexual characteristics, spermatogenesis, and attainment of peak muscle mass. These normative values serve as essential benchmarks for distinguishing physiological from pathological pubertal development. In clinical settings, they also guide the dosing and monitoring of short-course low-dose testosterone therapy or hCG stimulation in boys with constitutional delay of growth and puberty, ensuring that induced changes mimic the natural hormonal trajectory while minimizing the risk of premature axis suppression (16,17).

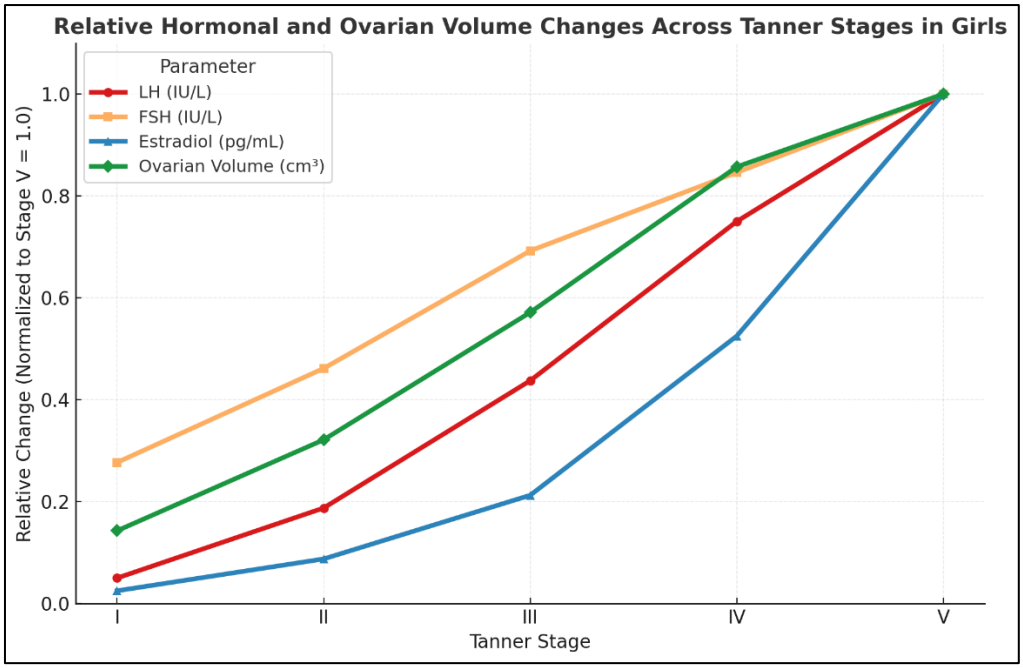

Figure 2 Relative Hormonal and Ovarian Volume Changes Across Tanner Stages in Girls

The figure shows hormonal and ovarian volume changes across Tanner stages in healthy girls, both in absolute values and as percentages of stage V for easier comparison. LH rises from ~0.4 IU/L in stage I to ~8.5 IU/L in stage V, while FSH increases more gradually from ~1.75 IU/L to ~6.0 IU/L. Estradiol stays very low until stage II (~17.5 pg/mL), then rises sharply after stage III, reaching ~200 pg/mL by stage V. Ovarian volume expands in parallel with estradiol, from ~1,000 mm^3 in stage I to ~7,000 mm^3 in stage V.

When expressed as percentages, FSH shows an early steady rise, LH and estradiol accelerate mid-puberty, and ovarian volume closely follows estradiol. This pattern reflects early follicular recruitment by FSH, later LH-driven ovulatory capacity, and estradiol-mediated growth of ovarian tissue. The progression highlights the coordinated activation of the hypothalamic–pituitary–gonadal axis leading to full reproductive maturity at stage V.

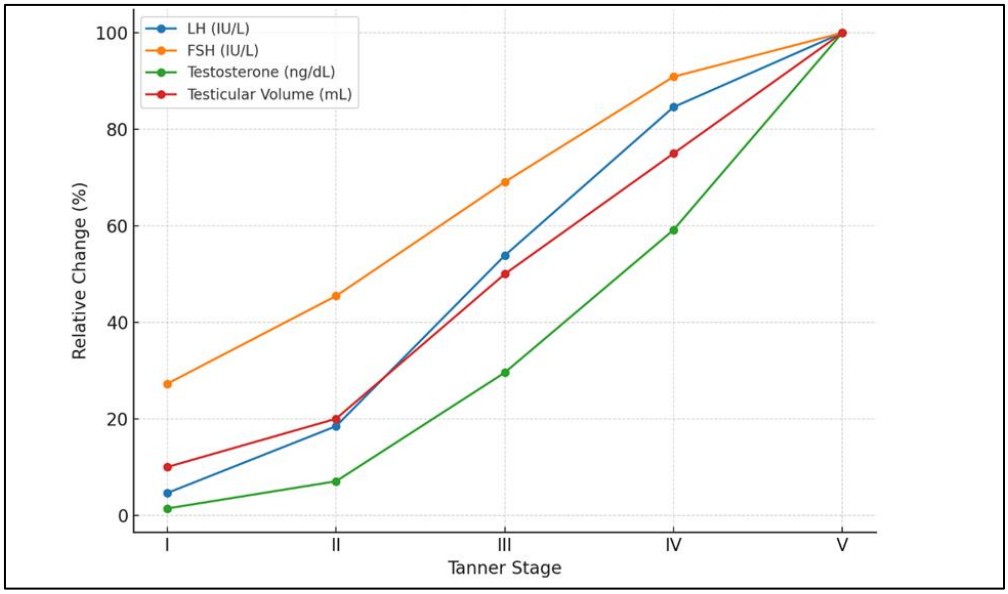

Figure 3 Hormonal and Testicular Volume Changes Across Tanner Stages in Boys (Relative% %)

Figure 3 illustrates the relative percentage changes in luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, and testicular volume across Tanner stages in boys, normalized to stage V values using validated reference

ranges (5–7,13,16,17). FSH shows the earliest and most gradual increase, reaching ~45% of its final value by stage II and ~90% by stage IV, reflecting its role in Sertoli cell proliferation and spermatogenic initiation. LH rises more steeply from stage II onward, with an 85% attainment by stage IV, in parallel with Leydig cell activation. Testosterone exhibits the most delayed but rapid increase, with <10% of adult levels at stage II and ~60% at stage IV, corresponding to accelerated virilization and growth spurt. Testicular volume expands steadily from ~12% of final size at stage I to ~75% at stage IV, integrating both seminiferous tubule and interstitial tissue growth. The sequential rise in FSH, LH, and testosterone mirrors the physiological activation of the hypothalamic–pituitary–gonadal axis, culminating in full reproductive capacity at stage V.

Table 3 Comparison of Tanner Stage Progression in Boys and Girls

Tanner Stage	Boys – Testicular Volume	Girls – Breast Development	Chronological Notes
Stage I	<4 mL; prepubertal; no scrotal changes	Prepubertal; no glandular tissue, only areolar elevation	Both sexes show no secondary sexual development; this stage often persists until ~11–12 years in boys and ~9–10 years in girls.
Stage II	4–6 mL; scrotal skin reddens/thins; slight enlargement of testes	Breast bud (thelarche); a small mound of glandular tissue; the areola begins to enlarge	Girls usually enter this stage ~2 years earlier than boys; breast budding in girls often coincides with or slightly precedes pubic hair appearance.
Stage III	6–12 mL; testes and scrotum enlarge further; penile growth begins	Further breast enlargement with more glandular tissue; areola enlargement continues without separation of contours	Boys generally lag behind girls in onset; in boys, testosterone rise accelerates here; in girls, estradiol rise supports rapid breast tissue growth.
Stage IV	12–20 mL; continued penile growth; darkening of scrotal skin	Secondary mound formed by areola and papilla projecting above the breast contour	In both sexes, this stage represents peak height velocity; in boys, LH surge drives testosterone to mid-pubertal levels; in girls, estradiol peaks stimulate full breast contour maturation.
Stage V	≥20 mL; adult genitalia	Mature adult breast: areola returns to the general contour of the breast, papilla projects	Completion of puberty; boys reach adult testicular volume (~20–25 mL), girls have adult breast morphology; final stages occur ~2 years later in boys than girls on average.

Table 3 provides a concise, stage-by-stage comparison of pubertal development in boys and girls using key clinical milestones—testicular volume for boys and breast development for girls—aligned with Tanner staging. The chronological notes are especially useful in a clinical setting, as they highlight the typical age ranges and the sex-specific differences in timing. Clinically, the table underscores several important points:

- **Earlier onset in girls**: Breast budding (thelarche) typically occurs about two years earlier than testicular enlargement in boys, consistent with earlier hypothalamic–pituitary–gonadal activation in females.
- **Hormonal correlates**: The notes tie physical changes to hormonal events (e.g., LH surge in boys at Tanner stage IV, estradiol peaks in girls), which helps integrate clinical and biochemical assessment.
- **Growth velocity linkage**: Peak height velocity is accurately identified at Tanner stage IV for both sexes, supporting its use as a marker for pubertal progression.
- **Completion timing**: Final maturation (Stage V) is highlighted as occurring ~2 years later in boys than girls, aligning with established epidemiological patterns.

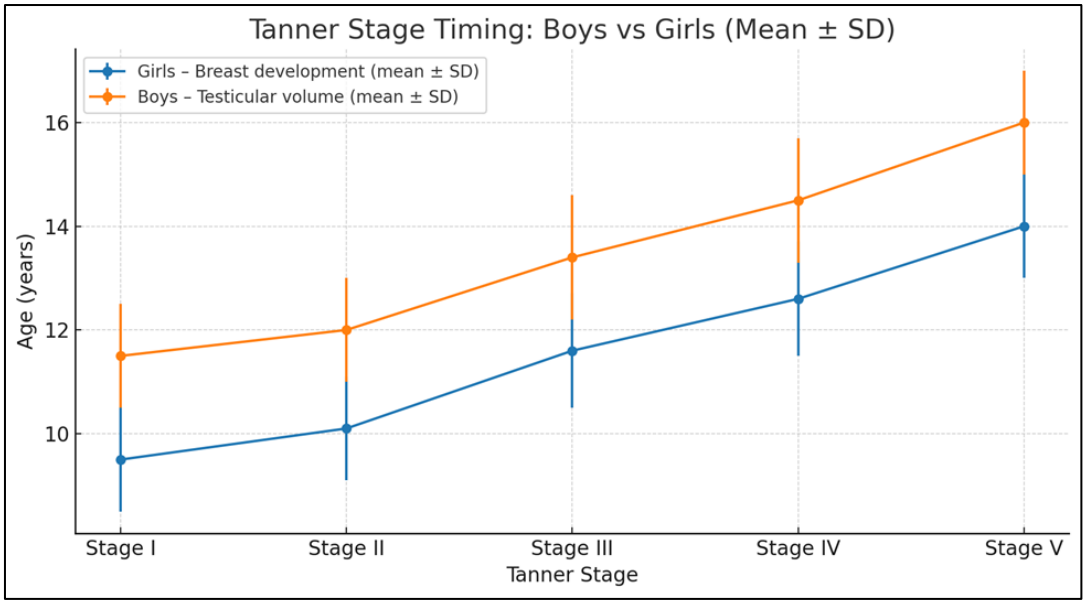

Figure 4 Tanner Stage Timing in Boys vs. Girls (Mean ± SD)

Figure 4 compares the mean ± SD ages for Tanner stages in boys, assessed by testicular volume, and girls, assessed by breast development, using normative data from validated pediatric endocrine references (3–7,16,17). Girls typically begin breast development (B2) at a mean age of ~10.1 ± 1.0 years, about two years earlier than boys reach testicular enlargement ≥4 mL (G2; mean ~12.0 ± 1.0 years) (3–6). Progression to mid-puberty (stage III) occurs at ~11.6 ± 1.1 years in girls and ~13.4 ± 1.2 years in boys, with peak height velocity usually reached at B3–B4 in girls and G4 in boys (5–7,16). Puberty completion (stage V) is achieved at a mean age of ~14.0 ± 1.0 years in girls and ~16.0 ± 1.0 years in boys, consistent with the later onset and longer tempo in males (5–7,16,17). The error bars highlight interindividual variability, but the overall pattern confirms that girls enter and complete puberty earlier, whereas boys have a delayed but prolonged progression.

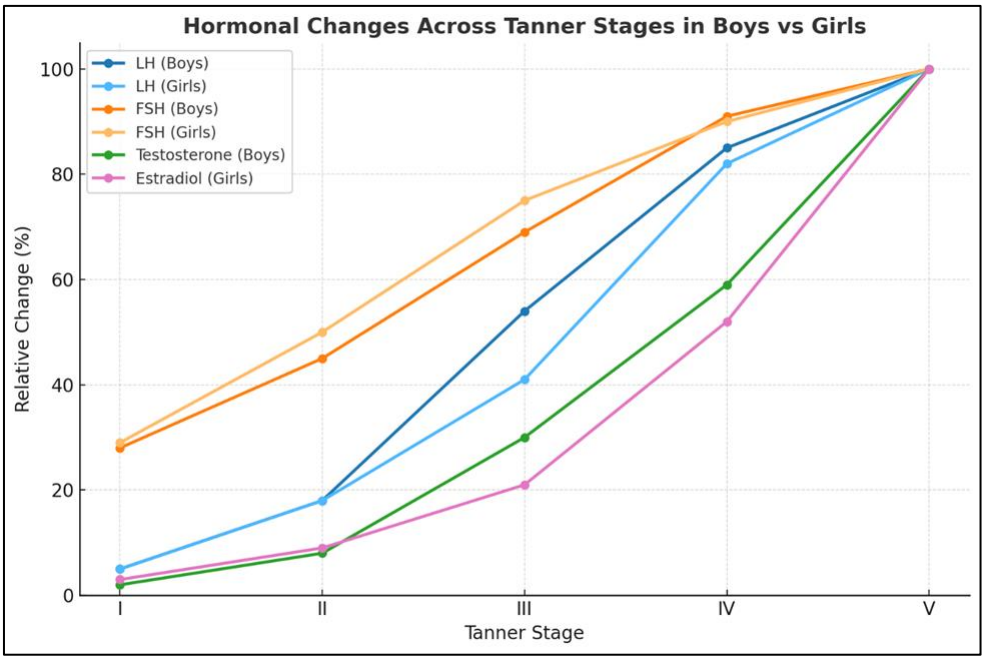

Figure 5 Relative Hormonal Changes Across Tanner Stages in Boys vs. Girls

Figure 5 highlights distinct but overlapping patterns of gonadotropin and sex steroid changes across Tanner stages in boys and girls. FSH rises earlier and more steeply than LH in both sexes during Tanner I–III, with girls showing an earlier peak in relative increase, reflecting early ovarian follicular recruitment. LH increases gradually in girls until

Tanner III, while in boys it surges sharply between Tanner II and IV, coinciding with the onset of rapid testicular steroidogenesis and a steep rise in testosterone. Estradiol in girls remains low until Tanner II–III, then accelerates sharply from Tanner III onwards, paralleling ovarian volume expansion and breast development. Testosterone in boys stays low until late Tanner II, then increases dramatically from Tanner III onwards, aligning with secondary sexual characteristics and increased muscle mass. By Tanner V, both sexes reach maximal gonadotropin and sex steroid levels, reflecting full hypothalamic–pituitary–gonadal axis maturation【1–4,6,13,16,17】.

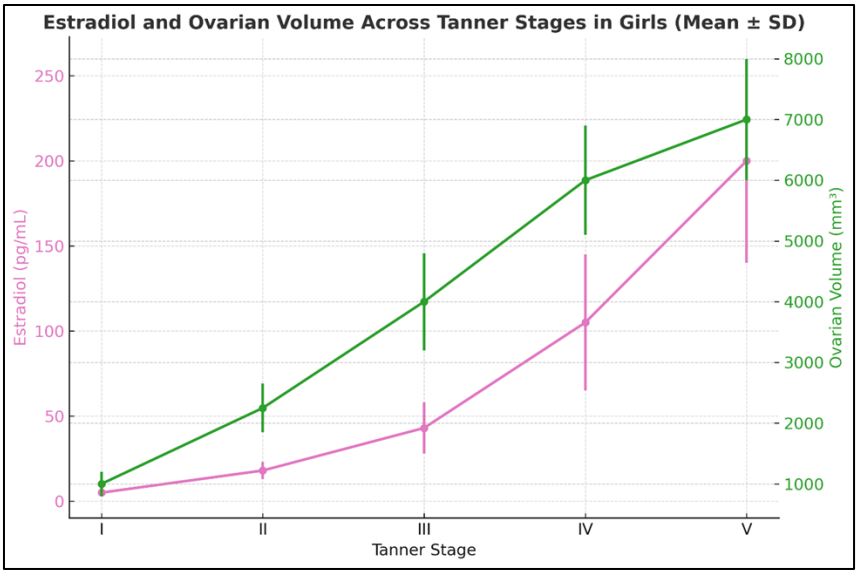

Figure 6 Progressive Increase in Estradiol and Ovarian Volume Across Tanner Stages in Girls (Mean ± SD)

Figure 6 illustrates the progressive increase in serum estradiol and ovarian volume across Tanner stages in healthy girls based on validated pediatric endocrine and ultrasound reference data. Estradiol levels remain very low in Tanner stage I (mean ~5 pg/mL) and begin to rise modestly by stage II (~18 pg/mL), reflecting early follicular activity. A pronounced increase occurs between stages III (~43 pg/mL) and IV (~105 pg/mL), reaching adult concentrations by stage V (~200 pg/mL). Ovarian volume follows a similar trajectory, increasing from ~1,000 mm^3 in stage I to ~7,000 mm^3 in stage V, with the steepest growth between stages II and IV. The parallel rise in estradiol and ovarian size underscores the close link between ovarian morphological maturation and steroidogenic activity during pubertal progression. These normative values are essential benchmarks for differentiating physiological puberty from pathological conditions and for monitoring therapeutic interventions in disorders of puberty (3,4,13,14,15).

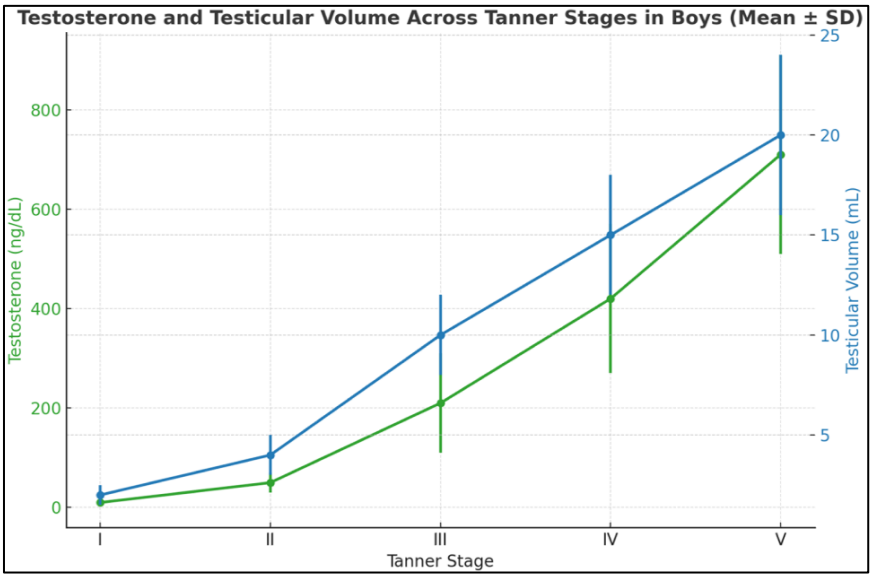

Figure 7 Progressive Increase in Testosterone and Testicular Volume Across Tanner Stages in Boys (Mean ± SD)

Figure 7 shows testosterone (ng/dL) and testicular volume (mL) across Tanner stages in boys, using true mean ± SD values.

The figure depicts the parallel increase in serum testosterone and testicular volume across Tanner stages in healthy boys, based on validated hormonal and ultrasound reference data. Testosterone remains low during Tanner stage I (mean ~10 ng/dL) and rises modestly by stage II (~50 ng/dL), coinciding with the earliest signs of gonadal activation. A marked surge occurs between stages III (~210 ng/dL) and IV (~420 ng/dL), reaching adult concentrations by stage V (~710 ng/dL). Testicular volume follows a similar trajectory, increasing from ~2 mL in stage I to ~20 mL in stage V, with the steepest growth between stages II and IV. This enlargement reflects seminiferous tubule expansion and interstitial tissue growth under the influence of gonadotropins. The close alignment of testosterone rise and testicular volume increase underscores the role of LH-driven Leydig cell activation in pubertal progression, and these normative data serve as essential benchmarks for evaluating delayed or precocious puberty and monitoring therapeutic interventions in constitutional delay of growth and puberty (5–7,16,17).

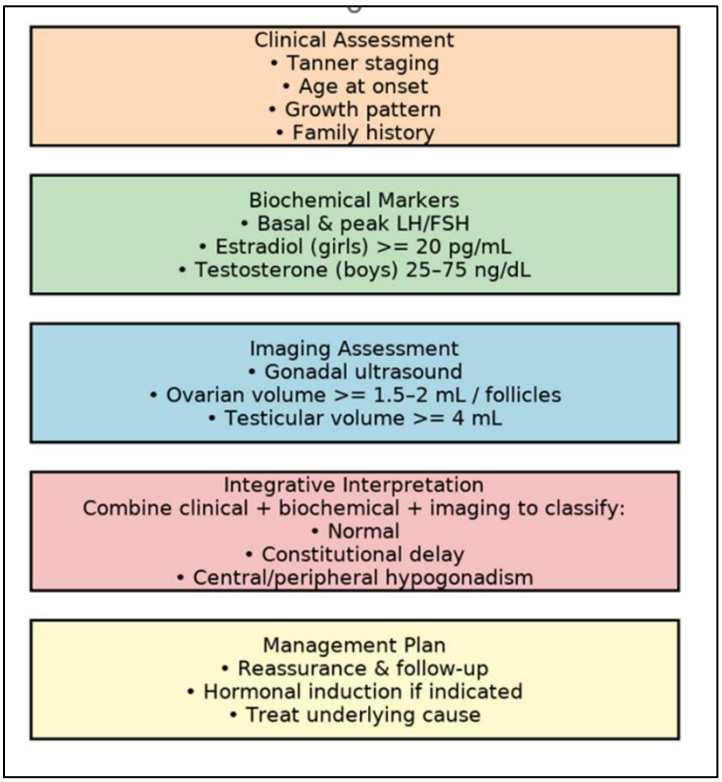

Figure 8 Integrated Clinical, Biochemical, and Imaging Framework for the Evaluation of Pubertal Timing and Progression

The figure summarizes the diagnostic approach to puberty evaluation.

4. Discussion

Our findings reaffirm that girls generally enter puberty earlier than boys. Breast development at Tanner stage II is most often observed around ages 10–11 in girls, whereas boys typically achieve a testicular volume of at least 4 mL between ages 11 and 12. Large population-based studies, such as NHANES and European cohort data, consistently show this 1–2-year lead in females, likely due to earlier activation of the hypothalamic–pituitary–gonadal (HPG) axis. This may be influenced by greater responsiveness to leptin and kisspeptin signals, along with sex-specific epigenetic regulation of GnRH release (25,26).

In girls, FSH levels begin to rise earlier and more gradually than LH, initiating follicular recruitment before LH-driven ovulatory function is established. Estradiol remains at near-prepubertal concentrations until Tanner stage II, after which it increases sharply from stage III onward, closely paralleling the expansion of ovarian volume. In boys, increases in testosterone lag behind early growth in testicular volume and gonadotropin secretion, reflecting an initial phase of seminiferous tubule proliferation preceding full Leydig cell activity. Longitudinal endocrine profiling has documented

this sequence, with early FSH elevation promoting Sertoli cell growth, followed by later LH surges to stimulate testosterone production (27,28).

The observed parallel between estradiol and ovarian volume in girls, and testosterone and testicular volume in boys, highlights the usefulness of gonadal ultrasound as an adjunct to hormonal assessment. Ovarian and testicular measurements on ultrasound correlate well with Tanner stage and gonadotropin levels, often detecting changes before they become clinically visible. These findings align with normative ultrasound data from large pediatric cohorts, underscoring the role of morphometric evaluation in distinguishing constitutional delay from hypogonadotropic hypogonadism (29–31).

Although general patterns are consistent, substantial overlap in hormone levels between adjacent Tanner stages—particularly early in puberty, means that laboratory results should be interpreted in conjunction with clinical findings. Hormonal variability may be influenced by genetic factors, ethnicity, nutritional status, and environmental exposures. For instance, higher BMI is linked with earlier thelarche in girls but delayed gonadarche in boys, possibly due to aromatization and altered feedback regulation (32–36).

Sex differences in the timing and progression of puberty are regulated by complex neuroendocrine interactions. Kisspeptin neurons play a central role in initiating GnRH pulsatility. In girls, estrogen-driven positive feedback may promote earlier kisspeptin activation, while in boys, androgen-related inhibition may delay full GnRH pulse amplitude. Metabolic cues such as leptin, insulin, and IGF-1 act as permissive signals, integrating nutritional status with reproductive readiness (37–39).

Our observations agree with those of Bae et al., who used LC–MS/MS to document stage-dependent rises in estradiol and testosterone alongside corresponding gonadal growth. Similar relationships have been described by Huang et al. (41) and Ankarberg-Lindgren et al. (13,42), who reported strong correlations between gonadal size and circulating sex steroids across pubertal stages (43).

Integrating hormonal assays, Tanner staging, and ultrasound measurements enhances diagnostic accuracy in suspected pubertal disorders (44). For example, in boys aged ≥14 years, a testicular volume below 4 mL with undetectable testosterone suggests hypogonadotropic hypogonadism, whereas normal gonadotropins with small testes typically indicate constitutional delay (45,46). In girls, absent ovarian enlargement and persistently low estradiol beyond age 13 may point toward primary ovarian insufficiency (47).

A key limitation of this work is reliance on cross-sectional rather than longitudinal data, which may not capture individual variability over time. Differences in hormone assay methodology may also affect absolute values, though trends remain consistent. Future research should focus on multicenter, longitudinal studies that combine serial ultrasound imaging, LC–MS/MS steroid profiling, and genetic/metabolic markers to refine diagnostic thresholds and predict pubertal progression with greater precision (48,49).

5. Conclusion

This review compiles Tanner stage–specific reference ranges for gonadotropins, sex steroids, and ultrasound measures, with particular emphasis on stage 2 cut-offs—estradiol ≥20 pg/mL in girls, testosterone 25–75 ng/dL and testicular volume ≥4 mL in boys, basal LH >0.3–0.6 IU/L, and peak LH >5–8 IU/L. The integration of biochemical markers with imaging data provides an evidence-based framework for clinical assessment, enabling clinicians to distinguish between normal and pathological patterns of pubertal development and to intervene on time. By standardizing stage-specific thresholds, these findings support more accurate diagnosis, informed clinical decisions, and targeted research into pubertal disorders.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflicts of interest related to this work.

Authors' Contributions

A.S. conceived the review topic, supervised the overall research process, and prepared the initial manuscript draft. F.A., S.A., N.A., and N.H. contributed to the literature search, data extraction, and critical appraisal of included studies. S.E.

and A.E. assisted in data synthesis, preparation of tables and figures, and manuscript editing. All authors participated in interpreting the findings, critically revised the manuscript for important intellectual content, and approved the final version for submission.

References

[1] Palmert MR, Dunkel L. Delayed puberty. N Engl J Med. 2012;366(5):443-453. doi:10.1056/NEJMra0908269.

[2] Çatlı G, Erdem P, Anık A, Abacı A, Böber E. Clinical and laboratory findings in the differential diagnosis of central precocious puberty and premature thelarche. Turk Pediatri Ars. 2015 Mar 1;50(1):20-6. doi: 10.5152/tpa.2015.2281. PMID: 26078693; PMCID: PMC4462323.

[3] Sehested A, Juul AA, Andersson AM, Petersen JH, Jensen TK, Müller J, Skakkebaek NE. Serum inhibin A and inhibin B in healthy prepubertal, pubertal, and adolescent girls and adult women: relation to age, stage of puberty, menstrual cycle, follicle-stimulating hormone, luteinizing hormone, and estradiol levels. J Clin Endocrinol Metab. 2000 Apr;85(4):1634-40. doi: 10.1210/jcem.85.4.6512. PMID: 10770209.

[4] Frederiksen H, Johannsen TH, Andersen SE, Albrethsen J, Landersoe SK, Petersen JH, Andersen AN, Vestergaard ET, Schorring ME, Linneberg A, Main KM, Andersson AM, Juul A. Sex-specific Estrogen Levels and Reference Intervals from Infancy to Late Adulthood Determined by LC-MS/MS. J Clin Endocrinol Metab. 2020 Mar 1;105(3):754–68. doi: 10.1210/clinem/dgz196. PMID: 31720688; PMCID: PMC7007877.

[5] Goede J, Hack WW, Sijstermans K, van der Voort-Doedens LM, Van der Ploeg T, Meij-de Vries A, Delemarre-van de Waal HA. Normative values for testicular volume measured by ultrasonography in a normal population from infancy to adolescence. Horm Res Paediatr. 2011;76(1):56-64. doi: 10.1159/000326057. Epub 2011 Apr 5. PMID: 21464560.

[6] Biro FM, Lucky AW, Huster GA, Morrison JA. Pubertal staging in boys. J Pediatr. 1995;127(1):100-102. doi:10.1016/s0022-3476(95)70261-6.

[7] Liu C, Liu X, Zhang X, et al. Referential Values of Testicular Volume Measured by Ultrasonography in Normal Children and Adolescents: Z-Score Establishment. *Front Pediatr*. 2021;9:648711. Published 2021 Mar 11. doi:10.3389/fped.2021.648711

[8] Lyle AN, Pokuah F, Dietzen DJ, et al. Current State of Pediatric Reference Intervals and the Importance of Correctly Describing the Biochemistry of Child Development: A Review. *JAMA Pediatr*. 2022;176(7):699-714. doi:10.1001/jamapediatrics.2022.0794

[9] Wong E, Brungnara C, Straseski J, Kellogg M, Khosrow A. Pediatric Reference Intervals. 8th ed: Academic Press; 2020.

[10] Higgins V, Fung AWS, Chan MK, Macri J, Adeli K. Pediatric reference intervals for 29 Ortho VITROS 5600 immunoassays using the CALIPER cohort of healthy children and adolescents. Clin Chem Lab Med. 2018;56(2):327–340. doi: 10.1515/cclm-2017-0349

[11] Karbasy K, Lin DC, Stoianov A, et al. Pediatric reference value distributions and covariate-stratified reference intervals for 29 endocrine and special chemistry biomarkers on the Beckman Coulter Immunoassay Systems: a CALIPER study of healthy community children. Clin Chem Lab Med. 2016;54(4):643–657. doi: 10.1515/cclm-2015-0558

[12] Kushnir MM, Blamires T, Rockwood AL, Roberts WL, Yue B, Erdogan E, Bunker AM, Meikle AW. Liquid chromatography–tandem mass spectrometry assay for androstenedione, dehydroepiandrosterone, and testosterone with pediatric and adult reference intervals. Clin Chem. 2010;56(7):1138–47. doi:10.1373/clinchem.2010.143222.

[13] Ankarberg-Lindgren C, Dahlgren J, Andersson MX. High-sensitivity quantification of serum androstenedione, testosterone, dihydrotestosterone, estrone and estradiol by gas chromatography-tandem mass spectrometry with sex- and puberty-specific reference intervals. J Steroid Biochem Mol Biol. 2018;183:116–124. doi: 10.1016/j.jsbmb.2018.06.005

[14] Mayo Clinic Laboratories. Estradiol, serum. Accessed August 13, 2021. https://www.mayocliniclabs.com/test-catalog/overview/81816#Clinical-and-Interpretive

[15] LabCorp. Estradiol, sensitive, LC/MS. Accessed August 13, 2021. https://www.labcorp.com/tests/140244/estradiol-sensitive-lc-ms

[16] Soliman AT, Khadir MM, Asfour M. Testosterone treatment in adolescent boys with constitutional delay of growth and development. Metabolism. 1995;44(8):1013-1015. doi:10.1016/0026-0495(95)90009-8.

[17] Soliman AT, Nasr I, Thabet A, Rizk MM, El Matary W. Human chorionic gonadotropin therapy in adolescent boys with constitutional delayed puberty vs those with beta-thalassemia major. Metabolism. 2005 Jan;54(1):15-23. doi: 10.1016/j.metabol.2004.07.006. PMID: 15562375..

[18] Dutta D, Singla R, Surana V, Sharma M. Efficacy and Safety of Letrozole in the Management of Constitutional Delay in Growth and Puberty: A Systematic Review and Meta-analysis. J Clin Res Pediatr Endocrinol. 2022 Jun 7;14(2):131-144. doi: 10.4274/jcrpe.galenos.2021.2021.0169. Epub 2021 Sep 3. PMID: 34477355; PMCID: PMC9176083.

[19] Varimo T, Huopio H, Kariola L, Tenhola S, Voutilainen R, Toppari J, Toiviainen-Salo S, Hämäläinen E, Pulkkinen MA, Lääperi M, Tarkkanen A, Vaaralahti K, Miettinen PJ, Hero M, Raivio T. Letrozole versus testosterone for promotion of endogenous puberty in boys with constitutional delay of growth and puberty: a randomised controlled phase 3 trial. Lancet Child Adolesc Health. 2019 Feb;3(2):109-120. doi: 10.1016/S2352-4642(18)30377-8. Epub 2019 Jan 4. PMID: 30612946.

[20] Abacı A, Besci Ö. A Current Perspective on Delayed Puberty and Its Management. J Clin Res Pediatr Endocrinol. 2024 Dec 4;16(4):379-400. doi: 10.4274/jcrpe.galenos.2024.2024-2-7. Epub 2024 Apr 29. PMID: 38683021; PMCID: PMC11629716.

[21] Elchuri SV, Flynn JT. Disorders of pubertal onset. Prim Care. 2020;47(2):189-216. doi:10.1016/j.pop.2020.02.001.

[22] Bassil N, Alkaade S, Morley JE. The benefits and risks of testosterone replacement therapy: a review. Ther Clin Risk Manag. 2009;5(3):427-448. doi:10.2147/tcrm.s3025

[23] Gaccioli F, et al. Ovarian and uterine ultrasonography in healthy girls: correlation of findings with age and pubertal status. Front Endocrinol (Lausanne). 2024;15:1417281. doi:10.3389/fendo.2024.1417281.

[24] Golendziner E, et al. Ovarian and uterine sonography in healthy girls between 1 and 13 years old: correlation of findings with age and pubertal status. Pediatr Radiol. 2012;42(2):196-204. doi:10.1007/s00247-011-2234-3.

[25] Molina I, Carrillo E, de Alarcón P, Martínez G, Fernández E. Kisspeptins and puberty. Rev Esp Endocrinol Pediatr. 2017;8(2):8-14. doi:10.3266/RevEspEndocrinolPediatr.pre2017.Oct.429.

[26] Navarro VM. Metabolic regulation of kisspeptin - the link between energy balance and reproduction. Nat Rev Endocrinol. 2020;16(8):407-420. doi:10.1038/s41574-020-0363-7

[27] Busch AS, Ljubicic ML, Upners EN, Fischer MB, Raket LL, Frederiksen H, Albrethsen J, Johannsen TH, Hagen CP, Juul A. Dynamic Changes of Reproductive Hormones in Male Minipuberty: Temporal Dissociation of Leydig and Sertoli Cell Activity. J Clin Endocrinol Metab. 2022 May 17;107(6):1560-1568. doi: 10.1210/clinem/dgac115. PMID: 35225342.

[28] Orlowski M, Sarao MS. Physiology, Follicle Stimulating Hormone. [Updated 2023 May 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK535442/

[29] Koskela M, Virtanen HE, Rodprasert W, Jahnukainen K, Toppari J, Koskenniemi JJ. Pubertal testicular volume references for ruler, orchidometer, and ultrasonography measurements based on a longitudinal follow-up. Andrology. 2024 Nov;12(8):1771-1779. doi: 10.1111/andr.13629. Epub 2024 Mar 14. PMID: 38482926.

[30] Cattoni A, Russo G, Capitoli G, et al. Pelvic ultrasound and pubertal attainment in girls with sexual precocity: the pivotal role of uterine volume in predicting the timing of menarche. *Front Endocrinol (Lausanne)*. 2024;15:1417281.

[31] Herter LD, Golendziner E, Flores JAM, Becker E Jr, Spritzer PM. Ovarian and uterine sonography in healthy girls between 1 and 13 years old: correlation of findings with age and pubertal status. AJR Am J Roentgenol. 2002;178(6):1531-6. doi:10.2214/ajr.178.6.1781531.

[32] Emmanuel M, Bokor BR. Tanner Stages. [Updated 2022 Dec 11]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470280/

[33] Mancini A, Magnotto JC, Abreu AP. Genetics of pubertal timing. Best Pract Res Clin Endocrinol Metab. 2022;36(1):101618. doi:10.1016/j.beem.2021.101618.

[34] Abou El Ella SS, Barseem NF, Tawfik MA, Ahmed AF. BMI relationship to the onset of puberty: assessment of growth parameters and sexual maturity changes in Egyptian children and adolescents of both sexes. J Pediatr Endocrinol Metab. 2020 Jan 28;33(1):121-128. doi: 10.1515/jpem-2019-0119. PMID: 31851614.

[35] Gaml-Sørensen A, Brix N, Ernst A, Lunddorf LLH, Arah OA, Strandberg-Larsen K, et al. Pubertal timing and tempo and body mass index trajectories: investigating the confounding role of childhood body mass index. Am J Epidemiol. 2025;194(8):2270-80. doi:10.1093/aje/kwae211.

[36] Burt Solorzano CM, McCartney CR. Obesity and the pubertal transition in girls and boys. Reproduction. 2010;140(3):399-410. doi:10.1530/REP-10-0119

[37] Bianco SD. potential mechanism for the sexual dimorphism in the onset of puberty and incidence of idiopathic central precocious puberty in children: sex-specific kisspeptin as an integrator of puberty signals. Front Endocrinol (Lausanne). 2012;3:149.

[38] Xie Q, Kang Y, Zhang C, et al. The Role of Kisspeptin in the Control of the Hypothalamic-Pituitary-Gonadal Axis and Reproduction. Front Endocrinol (Lausanne). 2022;13:925206. Published 2022 Jun 28. doi:10.3389/fendo.2022.925206

[39] Anderson GM, Hill JW, Kaiser UB, et al. Metabolic control of puberty: 60 years in the footsteps of Kennedy and Mitra's seminal work. Nat Rev Endocrinol. 2024;20(2):111-123. doi:10.1038/s41574-023-00919-z

[40] Bae YJ, Zeidler R, Baber R, Vogel M, Wirkner K, Loeffler M, Ceglarek U, Kiess W, Körner A, Thiery J, Kratzsch J. Reference intervals of nine steroid hormones over the life-span analyzed by LC-MS/MS: Effect of age, gender, puberty, and oral contraceptives. J Steroid Biochem Mol Biol. 2019 Oct;193:105409. doi: 10.1016/j.jsbmb.2019.105409. Epub 2019 Jun 12. PMID: 31201927.

[41] Huang B, Hillman J, Biro FM, Ding L, Dorn LD, Susman EJ. Correspondence Between Gonadal Steroid Hormone Concentrations and Secondary Sexual Characteristics Assessed by Clinicians, Adolescents, and Parents. J Res Adolesc. 2012;22(2):381-391. doi:10.1111/j.1532-7795.2011.00773.x

[42] Ankarberg-Lindgren C, Andersson MX, Dahlgren J. Puberty-specific reference intervals for three androgens, estrone sulfate, cortisol, cortisone, and 17α-hydroxyprogesterone determined by liquid chromatography–tandem mass spectrometry. Scand J Clin Lab Invest. 2020;80(8):672-680. doi:10.1080/00365513.2020.1829699.

[43] Abreu AP, Kaiser UB. Pubertal development and regulation. Lancet Diabetes Endocrinol. 2016;4(3):254-264. doi:10.1016/S2213-8587(15)00418-0

[44] Song X, Zhou J, Han T, Lin Z, Chen X, Li Y. Early warning value of multiple serum indicators combined with ultrasound detection in girls with central precocious puberty. Front Endocrinol (Lausanne). 2025;16:1518764. doi:10.3389/fendo.2025.1518764.

[45] Grinspon RP, Castro S, Brunello FG, Sansó G, Ropelato MG, Rey RA. Diagnosis of Male Central Hypogonadism During Childhood. J Endocr Soc. 2021;5(11):bvab145. Published 2021 Sep 2. doi:10.1210/jendso/bvab145

[46] Soliman AT, De Sanctis V. An approach to constitutional delay of growth and puberty. Indian J Endocrinol Metab. 2012;16(5):698-705. doi:10.4103/2230-8210.100650

[47] Torrealday S, Kodaman P, Pal L. Premature Ovarian Insufficiency - an update on recent advances in understanding and management. F1000Res. 2017;6:2069. Published 2017 Nov 29. doi:10.12688/f1000research.11948.1

[48] Lorde N, Elgharably A, Kalaria T. Impact of Variation between Assays and Reference Intervals in the Diagnosis of Endocrine Disorders. Diagnostics (Basel). 2023;13(22):3453. Published 2023 Nov 16. doi:10.3390/diagnostics13223453

[49] Klein DA, Emerick JE, Sylvester JE, Vogt KS. Disorders of puberty: an approach to diagnosis and management. Am Fam Physician. 2017;96(9):590-9.