

ORIGINAL RESEARCH

Tanner stages of thelarche in relation to anthropometric and ultrasound predictors in premenarcheal girls

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Abstract

Introduction: Thelarche is often the first sign of puberty initiation in girls. Although Tanner staging is widely used, it remains subjective. This study aimed to investigate the relationship between Tanner breast staging and anthropometric, breast ultrasound, and pelvic ultrasound parameters in healthy premenarcheal girls.

Material and Methods: A cross-sectional study was conducted at Vilnius University Hospital Santaros Clinics (September 2023–July 2025), including 251 premenarcheal girls aged 7–13 years. Breast development was staged using Tanner criteria. Anthropometric and ultrasound parameters were recorded, including body circumferences, skinfolds, uterine and ovarian dimensions, and breast tissue thickness. Logistic regression identified predictors of pubertal stage, and model performance was evaluated using ROC analysis.

Results: A total of 251 girls (mean age 10.5 ± 1.8 years) were included. Thelarche (Tanner B2) occurred at a mean age of 10.7 years. Anthropometric parameters varied significantly across Tanner stages, with body circumferences demonstrating very large effect sizes, whereas skinfold thickness showed only medium effects. Pelvic ultrasound parameters exhibited even stronger associations with Tanner staging. In multivariable analysis, uterine body length (OR=1.12 per mm, $p=0.004$), uterine thickness (OR=1.23 per mm, $p=0.003$), left ovarian follicle count (OR=1.39, $p=0.035$), and right ovarian length (OR=1.11, $p=0.013$) independently predicted Tanner B2–B3 status, while a higher number of right ovarian follicles was inversely associated (OR=0.72, $p=0.036$). The logistic model accurately distinguished Tanner B2–B3 from B1, demonstrating excellent discriminatory ability (AUC=0.924, 95% CI 0.889–0.959).

Conclusions: Anthropometric and particularly pelvic ultrasound parameters are strong indicators of thelarche and early pubertal maturation. Our findings support the use of pelvic ultrasound as an objective, non-invasive adjunct to Tanner staging, especially in situations where visual breast assessment may be uncertain.

Abbreviations: AUC, Area under the receiver operating characteristic curve; BMI, Body mass index; CI, Confidence interval; CPP, Central precocious puberty; ES, Effect size; HPG, Hypothalamic–pituitary–gonadal; IPT, Isolated premature thelarche; OR, Odds ratio; ROC, Receiver operating characteristic; SD, Standard deviation; SMR, Sexual Maturity Rating.

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KEYWORDS

anthropometric measurements, breast development, Tanner stages, Thelarche, ultrasound

1 | INTRODUCTION

Female breast development (thelarche) is a gradual process that becomes apparent during puberty, marking a significant transition from childhood to adulthood. It is often the first sign of puberty initiation in girls.¹ Differentiating isolated premature thelarche (IPT) from central precocious puberty is clinically important; however, objective tools for assessing early pubertal development remain limited.²

Several methods are available for assessing thelarche. The most widely used clinical approach is the Tanner staging system, which is based on somatoscopic assessment. Tanner staging, also known as sexual maturity rating (SMR), is an objective classification system that providers use to document and track the development and sequence of secondary sex characteristics of children during puberty.³ This method was developed by Marshall and Tanner during a longitudinal study conducted in England from the 1940s to the 1960s.³ According to the Tanner scale stages in breast evaluation, stage 1 (B1) represents the prepubertal state, characterized by the absence of glandular breast tissue, and stages 2 to 5 (B2–B5) represent the pubertal stages, marked by the presence of glandular breast tissue.⁴ Tanner breast stage 2 (B2) is characterized by the development of a small breast bud beneath the areola, with slight enlargement and elevation of the areola.⁵ The Tanner scale was originally based solely on visual inspection; however, contemporary clinical evaluation, which incorporates inspection and breast palpation, is now considered the gold standard for assessing breast development. Unfortunately, palpation is not used in routine anthropological studies of healthy individuals, when the aim is simply to determine the development of secondary sexual characteristics in a healthy population, and therefore an increase in fatty tissue in the breast area is often confused with the true onset of puberty. Additionally, although Tanner staging has been globally utilized for several decades, it remains a subjective method.

Over the last 20 years, several large-scale studies from high-income countries have reported an earlier onset of puberty in girls compared to historical norms, attributed to improved nutrition and environmental changes.^{5,6} The use of Tanner staging may encounter increased challenges as the prevalence of obesity rises among children and adolescents, potentially influencing the accuracy of assessment. Identifying glandular breast tissue vs. adipose tissue can be challenging during visual inspection and palpation. As a result, alternative assessment approaches are currently being explored.

Among available imaging methods, ultrasound represents a practical and non-invasive approach for assessing breast development. Ultrasound enables the identification of distinctive developmental

Key message

Simple anthropometric measurements, particularly age, body weight, inframammary chest circumference and waist circumference, accurately predict the onset of thelarche in premenarcheal girls. Pelvic ultrasound further improves objective pubertal assessment.

stages based on changes in the appearance and relative amounts of glandular, adipose, and fibrous tissues.⁷ Previous studies have demonstrated the potential of ultrasound-based breast assessment in relation to Tanner staging; however, available data remain heterogeneous and further validation in diverse populations is needed. Although several alternative methods for assessing breast development have been explored, including optical spectroscopy, mammography, magnetic resonance imaging (MRI), 3D imaging, and hormonal assessments, comparative data remain limited and their use in paediatric populations is restricted.^{8–10}

The aim of this study was to investigate the relationship between Tanner breast staging and breast ultrasonography, pelvic ultrasound findings, anthropometric characteristics, and the topography of subcutaneous adipose tissue in healthy premenarcheal girls.

2 | MATERIAL AND METHODS

2.1 | Study design

We performed a cross-sectional study in one clinical center (Vilnius University Hospital Santaros Klinikos). Participants were premenarcheal girls aged between 7 and 13 years. Potential participants were recruited from those receiving outpatient consultations at the Children's Consultative Outpatient Department or inpatient care at Vilnius University Hospital Santaros Klinikos, as well as from volunteers across Lithuania. They were informed about the study, and those who showed interest in participating were screened for eligibility.

Eligibility for inclusion in the study was determined based on predefined inclusion criteria. Those who met the criteria were approached with detailed information regarding the study's objectives, scope, data processing methods, and potential benefits. Both the participants and their parents or legal guardians were informed about the biomedical research procedures involved. Written informed consent was obtained from the parents or guardians of the study participants, while the minor participants provided assent. Consent and assent forms were signed before the inclusion of any participant in

the study. The recruitment period was from September 1, 2023, to July 31, 2025. Vilnius Regional Bioethics Committee approved the study under registration number 2023/5-1402-969 on May 9, 2023.

2.2 | Inclusion and exclusion criteria

Healthy premenarcheal girls between the ages of 7 and 13 years who gave written informed consent and agreed to participate in the study were included. Participants were excluded from the study based on any of the following criteria: failure to meet the inclusion criteria, refusal to participate in the study, missing or incomplete data in the medical records, presence of pathological and endocrinological conditions affecting the ovaries, uterus, breasts, or congenital abnormalities (such as defects in the formation of the uterus or vagina), girls who have already undergone menarche, a history of oncological diseases or other severe extragenital conditions that may influence the development of internal genital organs or breasts, diagnosis of established genetic disorders (e.g., trisomy 21 or Turner syndrome), residence in social welfare institutions, correctional facilities, or any other place of deprivation of liberty, parental employment within the healthcare institutions conducting the biomedical research, particularly if they are under the supervision of the researcher and/or absence of written informed consent from the parent(s)/legal guardian(s) or written assent from the participant.

2.3 | Study visits and data collection

All study participants were questioned and examined during one continuous visit. During this visit, the researcher interviewed all girls and their parents who met the inclusion criteria. Our data included demographic data, anthropometric data, ultrasound examination data, and the presence of chronic health conditions and medications. Breasts were evaluated according to the Tanner stages as follows: breast1 (B1): no glandular tissue—areola follows the skin contours of the chest (prepubertal), breast2 (B2)—breast bud forms, with small area of surrounding glandular tissue, areola begins to widen, breast3 (B3)—breast begins to become more elevated, and extends beyond the borders of the areola, which continues to widen but remains in contour with surrounding breast, breast4 (B4)—increased breast sizing and elevation, areola and papilla form a secondary mound projecting from the contour of the surrounding breast, breast5 (B5)—breast reaches final adult size, areola returns to contour of the surrounding breast, with a projecting central papilla.¹¹ Tanner breast staging was performed independently by two experienced clinicians using standardized visual assessment procedures without palpation. In cases of uncertainty, assessments were discussed jointly to ensure consistent classification. Although inter-rater reliability was not formally quantified, all evaluations were conducted according to predefined clinical criteria.

The following anthropometric measurements were evaluated and obtained using standard methods for individuals wearing light

clothing without shoes: body weight, height, body mass index [BMI], circumferences of the chest, waist, hip, triceps, mid-thigh, and skinfold thicknesses in subscapular, triceps, abdominal, suprailiac, mid-thigh, and calf areas.^{12,13} Height was measured in the standing position with a MARSDEN Portable Stadiometer (UK) (measurement accuracy ± 5 mm). Three height measurements were taken, and the average of these measurements was recorded in the questionnaire. To obtain the most accurate data, three measurements were chosen, the difference between which was less than 5 mm. Body mass was measured in light clothing with an electronic scale (MARSDEN, UK) with an accuracy of 0.1 kg. The scales were regularly calibrated with standard weights. BMI was calculated by the following formula: $BMI = \text{body mass (kg)} / \text{height (m)}^2$.¹⁰ The definitions from the International Obesity Task Force were used for assessing the BMI of the study subjects.^{14,15} For measuring the thickness of skinfolds, a Holtain caliper was used (Siber Hegner GMP, Switzerland; scale 40 mm, pitch 0.2 mm, pleat pressing pressure 10 g/mm², error 0.1 cm). To obtain the most accurate final value, three measurements were taken, and the median was selected as the result.

Pelvic and breast ultrasonography was performed on all the study subjects. Ultrasound examinations were performed by two experienced sonographers using a standardized protocol. Although inter-rater reliability was not formally assessed, all measurements were obtained according to predefined criteria to ensure consistency. The sonographers were not blinded to the clinical Tanner stage at the time of examination. The examinations were performed using a Mindray Resona 7 ultrasound machine with a convex 1–6 MHz probe for pelvic scan and a linear 8–14 MHz probe for breasts. The pelvic transabdominal ultrasonography findings for each case were recorded, including the following measurements: total uterine length, uterine body and cervical length, width, thickness, endometrial thickness, ovarian size (length, width, and thickness), ovarian volume, and the number of follicles observed in a single field of view. Ultrasound evaluations of both breasts were performed to assess the following parameters: thickness at the nipple, thickness in the quadrants, and the presence and characteristics of ducts at the nipple.

Ultrasound was used to assess breast tissue characteristics, including glandular, adipose, and fibrous components. US B0 was defined as a small hypoechoic retro-areolar mass, whereas US B1 was characterized by a hyperechoic area with or without a small hypoechoic mass.⁷ According to previously published criteria, US B0 and US B1 were considered prepubertal ultrasound stages and US B2 and US B3 represented pubertal breast development. Ultrasound stages were evaluated in relation to Tanner breast staging rather than as a measure of agreement with Tanner classification. US B2 was characterized by the presence of hyperechoic retro-areolar glandular tissue with a round or star-shaped hypoechoic center, representing the onset of puberty. US B3 was characterized by a larger spider-shaped hypoechoic center, representing further progression of pubertal breast development.

Because distinguishing the onset of breast development from Tanner stage B1 can be challenging in some girls, we grouped

participants into Tanner breast stage 1 (B1) and a combined early pubertal category (B2–B3) to minimize misclassification and ensure consistent staging.

2.4 | Statistical analysis

Statistical analyses were performed using R statistical software version 4.4.3, RStudio 2024.12.1, IBM SPSS Statistics version 23, and G*Power 3.1.9.4. Quantitative variables are presented as mean $\pm$ standard deviation (SD), median, and interquartile range (Q1–Q3), whereas categorical variables are presented as absolute numbers and percentages. Normality was assessed using the Shapiro–Wilk test. Group comparisons were performed using the Mann–Whitney *U* test, Kruskal–Wallis test, Welch's *F* test, Pearson's chi-square test, or Fisher's exact test, as appropriate. Effect sizes were estimated using omega squared (ω^2), rank epsilon squared ($\epsilon^2_{\text{ordinal}}$), rank-biserial correlation coefficient, and Cramer's *V*, depending on variable type and distribution. Statistical significance was defined as $p < 0.05$.

We constructed a binary logistic regression model to estimate the odds of belonging to Tanner breast stages B2–B3 vs. B1. Tanner breast stage (B1 vs. B2–B3) was used as the dependent outcome variable. All anthropometric and ultrasound-derived variables were initially entered into the regression analysis. Multiple candidate models were subsequently evaluated, and the final model was selected based on Akaike information criterion (AIC), Bayesian information criterion (BIC), overall model fit, and clinical interpretability. Because age is intrinsically related to pubertal progression and highly correlated with several anthropometric and ultrasound variables, it was not included in the final multivariable model to avoid overadjustment and multicollinearity. The final model represented the optimal balance between statistical performance and clinical plausibility. We considered the relationships between variables to be statistically significant when the *p*-value was less than 0.05 ($p < 0.05$), and the statistical power of the test $1 - \beta$ was equal to 0.80 ($1 - \beta = 0.80$).

A post hoc sample size estimation for the logistic regression model was performed using the Demidenko variance-corrected method (Wald's *Z*-test). The required sample size was estimated at 238 participants to achieve 80% statistical power at $\alpha = 0.05$. The final study sample of 251 participants therefore met the estimated requirement for multivariable logistic regression analysis.

3 | RESULTS

A total of 300 premenarcheal girls, aged 7–13 years, were invited to participate. Parental consent and assent from the participant were received prior to examination from 251 (83.7%) girls.

By using Probit Analysis, we estimated age-related probabilities, which showed a sequential pattern of breast development. The probability of being in Tanner B1 stage was almost 100%

before the age of 9, but it decreased rapidly thereafter, with the estimated midpoint of transition to Tanner B2 occurring at 10.7 years. The probability of Tanner B2 increased after 10 years, peaked between 11 and 12 years, and subsequently declined. The midpoint for the transition from Tanner B2 to B3 was identified at 12.2 years, indicating that by this age, most participants had progressed beyond B2. Figure 1 presents the distribution of subjects by age and Tanner stage, whereas Figure 2 illustrates the corresponding reference ranges.

The study groups' demographic and anthropometric parameters are presented in Table 1. Statistically significant differences were observed between the groups in age, height, weight, and the breast-to-chest circumference ratio and difference ($p < 0.001$, respectively). The latter parameters were additionally assessed to provide a more detailed evaluation of chest and breast development, as they are not routinely included in standard anthropometric assessments. All measured body circumferences showed statistically significant differences between groups according to Tanner stages ($p < 0.001$). A similar pattern was observed for skinfold thickness measurements, with the subscapular and abdominal skinfolds showing the most pronounced differences (ES = 0.26, 95% CI 0.39–0.12, $p < 0.001$ and ES = 0.28, 95% CI 0.41–0.15, $p < 0.001$, respectively).

Circumference parameters showed very large effect sizes (ES range: 0.48–0.97), indicating strong associations with Tanner stage progression. In contrast, all skinfold thickness parameters demonstrated medium ES values (subscapular: ES = 0.28 [95% CI 0.40 to 0.14]; biceps: ES = 0.26 [95% CI 0.39 to 0.12]; thigh: ES = 0.24 [95% CI 0.37 to 0.10]; medial-calf: ES = 0.26 [95% CI 0.38 to 0.12]), suggesting more moderate changes in subcutaneous fat distribution with advancing pubertal development. However, the abdominal skinfold thickness exhibited a large ES (ES = 0.32 [95% CI 0.44 to 0.18]), indicating a comparatively stronger pubertal-related increase in this region.

All evaluated pelvic ultrasound gynecological parameters showed statistically significant differences between the groups (Table 2). When evaluating breast ultrasound data, we observed a statistically significant difference in breast tissue thickness at the nipple level between the study groups, based on Tanner stage ($p < 0.001$). However, when assessing subcutaneous tissue thickness in individual breast quadrants, no significant differences were found, as only 42 participants had measurements for this parameter, which can be obtained once breast development begins.

The uterocervical ratio was significantly higher in Tanner stage 2 compared with Tanner stage 1 (median: 1.39 vs. 0.82, $p < 0.001$). This finding indicates that during puberty, the uterine body grows more rapidly than the cervix. The ES was very large (ES = 0.68, 95% CI 0.75–0.60), suggesting that this parameter may be one of the most reliable ultrasound markers of pubertal progression (Figure 3). ROC analysis demonstrated good discriminatory performance of the uterocervical ratio for differentiating Tanner B1 from B2–B3 stages, with an AUC of 0.842 (95% CI 0.791–0.893). The optimal cut-off value was 0.469, yielding a sensitivity of 83.6%

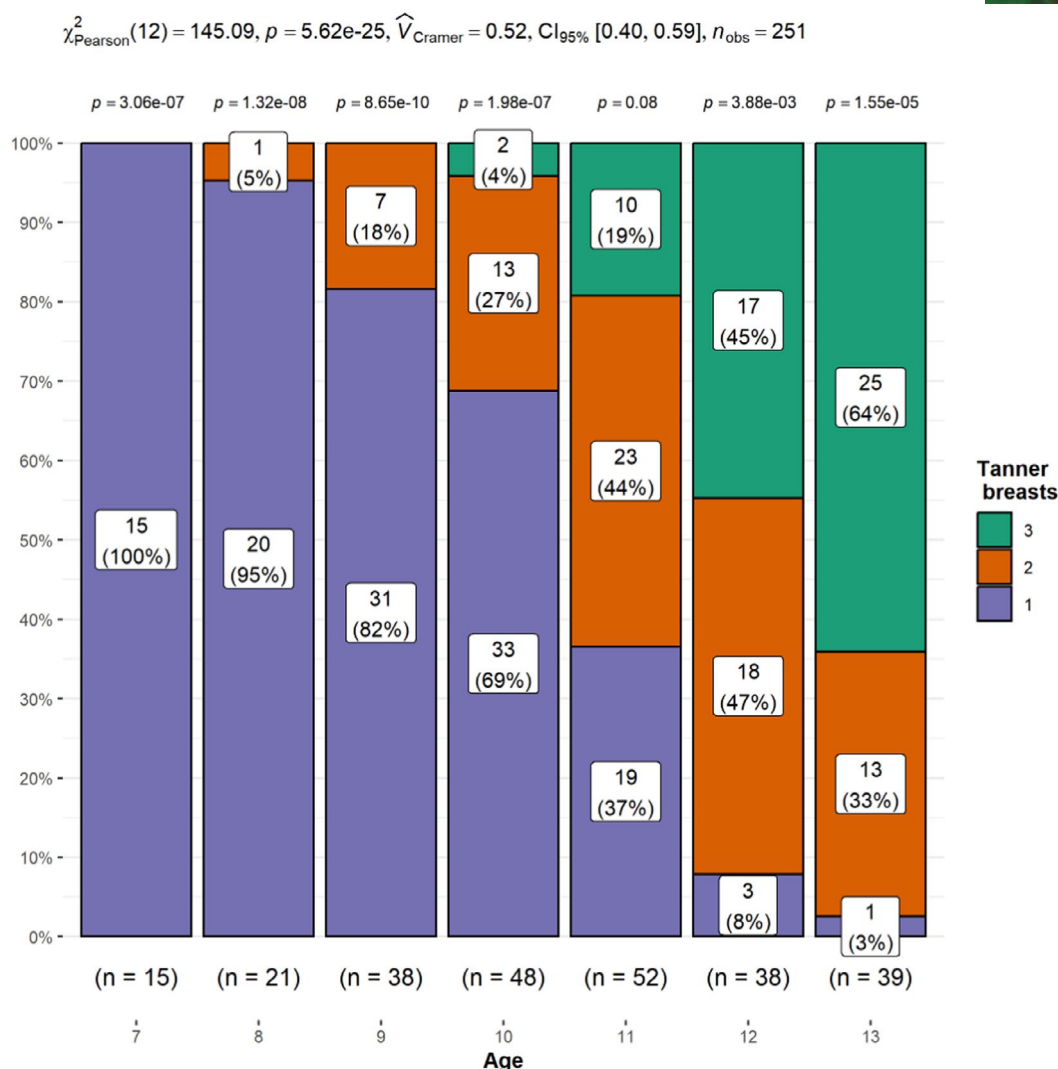


FIGURE 1 Distribution of subjects according to age and Tanner breast stage. Stacked bar plots demonstrate the proportion of girls classified as Tanner breast stages B1–B3 across age groups. Numbers within bars represent absolute counts and percentages within each age category. Pearson's chi-square test demonstrated a significant association between age and Tanner stage distribution ($\chi^2 = 145.09, p < 0.001$), with a large effect size (Cramer's $V = 0.52$). p -values above individual bars indicate comparisons between adjacent age groups.

and specificity of 76.7%. Detailed ROC threshold analysis and additional model diagnostics are provided in the [Supplementary Material](#).

Results of the logistic regression analysis are presented in [Table 3](#). The model estimated the probability of breast development being at Tanner stages B2 or B3 compared with B1. Tanner B2–B3 was associated with greater uterine body length (OR=1.12 per mm, $p=0.004$), increased uterine thickness (OR=1.23 per mm, $p=0.003$), a higher follicle count in the visual field of the left ovary (OR=1.39 per follicle, $p=0.035$), and increased right ovarian length (OR=1.11 per mm, $p=0.013$). In contrast, a greater number of right ovarian follicles was associated with lower odds of Tanner B2–B3 (OR=0.72 per follicle, $p=0.036$).

A logistic regression model was developed to estimate the probability of girls being in Tanner breast stages B2–B3 rather than B1, based on ultrasound-derived parameters. The final equation was $\text{Ln}[P(\text{B2–B3})/P(\text{B1})] = 0.11 \times \text{uterine body length} + 0.20 \times \text{uterine}$

$\text{thickness} + 0.33 \times \text{left ovarian follicle count} + 0.10 \times \text{right ovarian length} - 0.32 \times \text{right ovarian follicle count} - 6.84$. All predictors were statistically significant ($p < 0.05$), with positive coefficients indicating increasing odds of being in Tanner B2–B3, except for the number of right ovarian follicles, which showed a negative association. Using logistic transformation, the predicted probabilities were obtained as $P(\text{B2–B3}) = e^z / (1 + e^z)$ and $P(\text{B1}) = 1 / (1 + e^z)$. The overall model fit was strong ($\chi^2(5) = 144.09, p < 0.001$; McFadden pseudo- $R^2 = 0.49$), indicating that nearly half of the variance in breast developmental stage was explained by the included predictors. The model also demonstrated excellent diagnostic performance, with an AUC of 0.924 (95% CI: 0.889–0.959, $p < 0.001$) ([Figure 4](#)), high specificity (89%) and moderate sensitivity (83%). Overall accuracy reached 86%, with a positive predictive value of 89% and a negative predictive value of 83%. Together, these findings indicate that the model may serve as a useful adjunct in the ultrasound-based assessment of pubertal breast development.

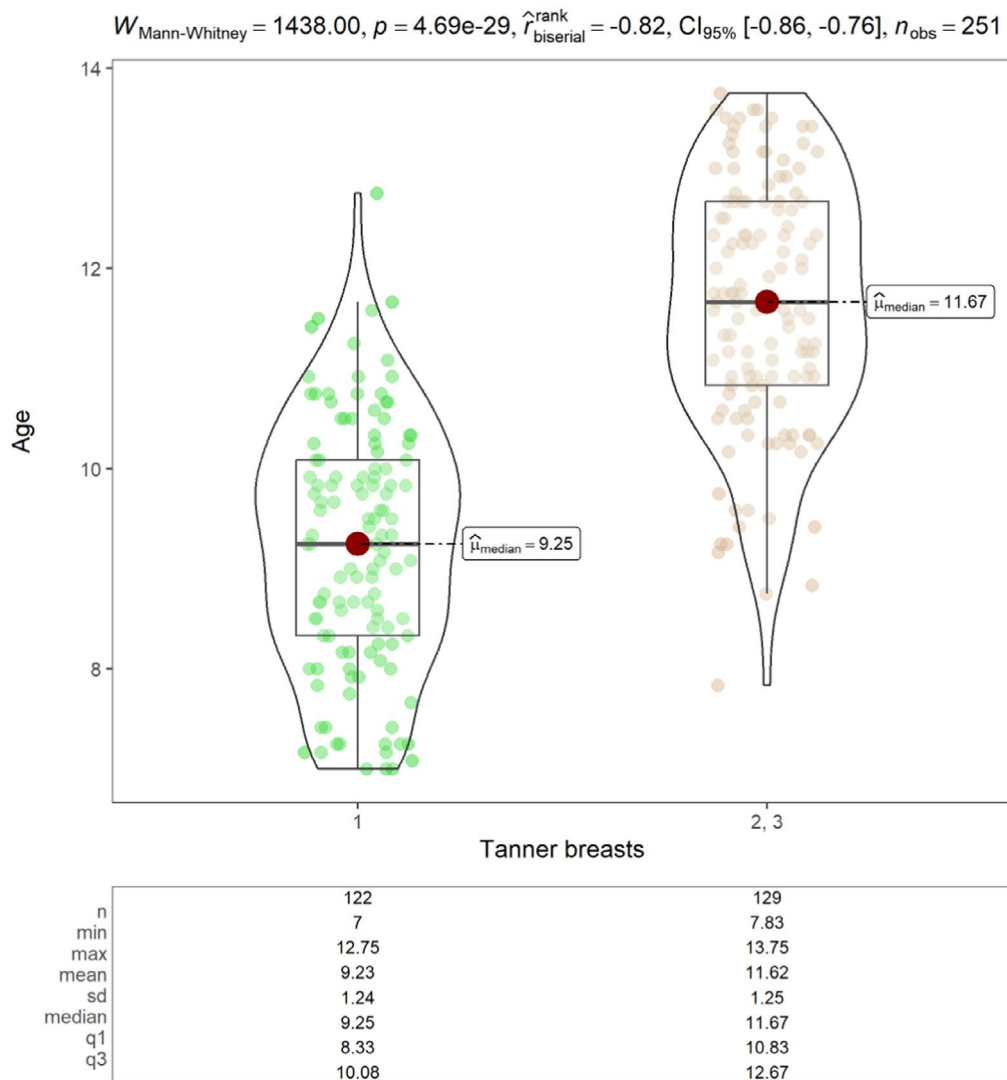


FIGURE 2 Age distribution according to Tanner breast stage groups. Violin plots with embedded boxplots demonstrate the age distribution of girls classified as Tanner breast stage B1 vs. combined Tanner stages B2–B3. Red dots indicate group medians. Numbers below the x-axis represent sample sizes for each group. The Mann–Whitney *U* test demonstrated a significant age difference between groups ($W = 1438.00, p < 0.001$), with a large effect size (rank-biserial correlation = 0.82).

4 | DISCUSSION

This study provides new insights into the assessment of pubertal development in premenarcheal girls by integrating anthropometric data with detailed pelvic ultrasound measurements.

We observed thelarche (Tanner B2) at a mean age of approximately 10.7 years. This is a young age, but our results are consistent with trends reported in recent literature. Recent evidence suggests a secular trend toward earlier pubertal onset, with the age at thelarche decreasing by approximately 3 months per decade since 1977.⁵

Anthropometric parameters demonstrated significant differences across Tanner stages, with body circumferences showing very large effect sizes (ES range 0.48–0.60, all $p < 0.001$). In contrast, all skinfold thickness measures showed only medium effect sizes, except abdominal skinfold thickness (ES = 0.32, 95% CI 0.44 to 0.18). These findings indicate that body circumferences correlate more

strongly with pubertal progression than subcutaneous fat distribution. This is biologically plausible, as body circumferences reflect the combined contribution of skeletal structure, muscle mass, and subcutaneous adipose tissue. During the premenarchal period, girls experience a pronounced acceleration in skeletal and muscular development, corresponding to a linear growth or “stretching” phase, in which increases in body size are driven predominantly by active tissue rather than passive fat mass. In contrast, sex-specific or sex-related fat accumulation typically intensifies during the later stages of puberty, commonly described as the “rounding” phase, which may explain the weaker associations observed between skinfold thickness and pubertal progression at this stage.¹¹

Ultrasound-derived uterine and ovarian parameters showed even stronger associations with breast maturation. Uterine body length and uterine thickness were markedly greater in Tanner B2–B3 compared with B1 and remained robust independent predictors

TABLE 1 Study groups' baseline demographic and anthropometric parameters.

Characteristic	N	Overall N = 251 ^a	Tanner breast		p-value ^b	Effect size (ES) (95% CI) ^c	Interpretation ^d
			B1	B2–B3			
			N = 122	N = 129			
Age (years)	251				<0.001	−0.82 (−0.86, −0.76)	Very large
N (non-missing)		251	122	129			
Median (Q1, Q3)		10.50 (9.25, 11.75)	9.25 (8.33, 10.08)	11.67 (10.83, 12.67)			
Mean (SD)		10.46 (1.73)	9.23 (1.24)	11.62 (1.25)			
Min, Max		7.00, 13.75	7.00, 12.75	7.83, 13.75			
Age (years)	251				<0.001	0.67 (0.53, 0.79)	
7		15 (6.0%)	15 (12%)	0 (0%)			
8		21 (8.4%)	20 (16%)	1 (0.8%)			
9		38 (15%)	31 (25%)	7 (5.4%)			
10		48 (19%)	33 (27%)	15 (12%)			
11		52 (21%)	19 (16%)	33 (26%)			
12		38 (15%)	3 (2.5%)	35 (27%)			
13		39 (16%)	1 (0.8%)	38 (29%)			
Height (cm)	251				<0.001	−0.74 (−0.80, −0.67)	Very large
N (non-missing)		251	122	129			
Median (Q1, Q3)		147 (138, 155)	139 (134, 146)	153 (148, 160)			
Weight (kg)	251				<0.001	−0.70 (−0.76, −0.61)	Very large
N (non-missing)		251	122	129			
Median (Q1, Q3)		37 (30, 45)	31 (27, 38)	43 (37, 50)			
Breast circumference/chest circumference	251				<0.001	−0.97 (−0.97, −0.95)	Very large
N (non-missing)		251	122	129			
Median (Q1, Q3)		1.03 (1.00, 1.08)	1.00 (1.00, 1.00)	1.08 (1.05, 1.11)			
Breast circumference-chest circumference (cm)	251				<0.001	−0.97 (−0.98, −0.96)	Very large
N (non-missing)		251	122	129			
Median (Q1, Q3)		2.0 (0.0, 6.0)	0.0 (0.0, 0.0)	6.0 (4.0, 8.0)			
Waist circumference (cm)	251				<0.001	−0.55 (−0.64, −0.44)	Very large
N (non-missing)		251	122	129			
Median (Q1, Q3)		59 (54, 65)	55 (52, 60)	62 (58, 69)			
Hip circumference (cm)	250				<0.001	−0.59 (−0.67, −0.49)	Very large
N (non-missing)		250	122	128			

(Continues)

TABLE 1 (Continued)

Characteristic	N	Overall N = 251 ^a	Tanner breast		p-value ^b	Effect size (ES) (95% CI) ^c	Interpretation ^d
			B1	B2-B3			
Median (Q1, Q3)							
Upper arm circumference (cm)	251	76 (69, 83)	70 (64, 76)	81 (75, 88)	<0.001	-0.48 (-0.58, -0.36)	Very large
N (non-missing)		251	122	129			
Median (Q1, Q3)		22.0 (20.0, 25.0)	21.0 (19.0, 23.0)	24.0 (21.0, 27.0)			
Thigh circumference (cm)	250	250	122	128	<0.001	-0.60 (-0.68, -0.49)	Very large
N (non-missing)		44 (40, 49)	41 (37, 45)	48 (44, 53)			
Median (Q1, Q3)							
Subscapular skinfold (mm)	251	251	122	129	<0.001	-0.28 (-0.40, -0.14)	Medium
N (non-missing)		251	122	129			
Median (Q1, Q3)		8 (5, 12)	7 (5, 11)	10 (7, 17)			
Biceps skinfold (mm)	251	251	122	129	<0.001	-0.26 (-0.39, -0.12)	Medium
N (non-missing)		13 (9, 18)	12 (9, 15)	14 (10, 20)			
Median (Q1, Q3)							
Abdominal skinfold (mm)	251	251	122	129	<0.001	-0.32 (-0.44, -0.18)	Large
N (non-missing)		11 (7, 19)	9 (6, 15)	14 (9, 24)			
Median (Q1, Q3)							
Thigh skinfold (mm)	251	251	122	129	<0.001	-0.24 (-0.37, -0.10)	Medium
N (non-missing)		19 (14, 25)	17 (13, 22)	20 (15, 27)			
Median (Q1, Q3)							
Medial-calf skinfold (mm)	251	251	122	129	<0.001	-0.26 (-0.38, -0.12)	Medium
N (non-missing)		14.0 (11.0, 18.0)	12.0 (10.0, 15.0)	15.0 (11.0, 20.0)			
Median (Q1, Q3)							

^aData are presented as median (Q1–Q3), mean (SD), or n (%), as appropriate.^bGroup comparisons were performed using the Kruskal–Wallis test, Pearson's chi-squared test, or Fisher's exact test, as appropriate.^cEffect size (ES) was expressed as rank-biserial correlation coefficient for continuous variables and Cramer's V for categorical variables.^dTanner breast stages B2 and B3 were combined for comparative analyses.

TABLE 2 Study groups' ultrasound parameters.

		Tanner breast					
		Overall	B1	B2-B3			
Characteristic	N	N = 251 ^a	N = 122	N = 129	p-value ^b	Effect size (ES) (95% CI) ^c	Interpretation ^d
Pelvic ultrasound. Gynecological parameters							
Uterine length (mm)	251				<0.001	−0.77 (−0.82, −0.70)	Very large
N (non-missing)		251	122	129			
Median (Q1, Q3)		36 (28, 49)	29 (25, 35)	48 (40, 59)			
Uterine cervical length (mm)	251				<0.001	−0.35 (−0.47, −0.22)	Large
N (non-missing)		251	122	129			
Median (Q1, Q3)		18.7 (15.7, 21.8)	17.1 (14.9, 20.5)	20.3 (17.0, 22.9)			
Uterine body length (mm)	251				<0.001	−0.79 (−0.84, −0.73)	Very large
N (non-missing)		251	122	129			
Median (Q1, Q3)		18 (12, 30)	12 (9, 16)	29 (21, 37)			
Uterine body width (mm)	251				<0.001	−0.79 (−0.84, −0.73)	Very large
N (non-missing)		251	122	129			
Median (Q1, Q3)		19 (14, 28)	14 (13, 17)	26 (21, 33)			
Uterine thickness (mm)	251				<0.001	−0.79 (−0.84, −0.73)	Very large
N (non-missing)		251	122	129			
Median (Q1, Q3)		9.4 (6.5, 16.5)	6.6 (5.7, 8.3)	15.9 (11.4, 19.9)			
Thickness of endometrium (mm)	215 ^e				<0.001	−0.77 (−0.82, −0.70)	Very large
N (non-missing)		215	94	121			
Median (Q1, Q3)		2.10 (1.40, 3.80)	1.40 (1.10, 1.80)	3.30 (2.20, 5.00)			
Uterocervical ratio	251				<0.001		
Body = cervix		12 (4.8%)	7 (5.7%)	5 (3.9%)			
Body > cervix		113 (45%)	17 (14%)	96 (74%)			
Cervix > body		126 (50%)	98 (80%)	28 (22%)			
Uterocervical ratio	251				<0.001	−0.68 (−0.75, −0.60)	Very large
N (non-missing)		251	122	129			
Median (Q1, Q3)		0.98 (0.64, 1.55)	0.66 (0.57, 0.92)	1.42 (1.06, 1.79)			
Length of left ovary (mm)	246				<0.001	−0.50 (−0.60, −0.38)	Very large
N (non-missing)		246	120	126			
Median (Q1, Q3)		25 (19, 29)	21 (16, 27)	28 (24, 32)			
Width of left ovary (mm)	246				<0.001	−0.53 (−0.63, −0.42)	Very large
N (non-missing)		246	120	126			
Median (Q1, Q3)		12.5 (10.1, 15.1)	11.1 (9.1, 12.7)	14.2 (11.8, 16.6)			

(Continues)

Characteristic	N	Overall N = 251 ^a	Tanner breast		p-value ^b	Effect size (ES) (95% CI) ^c	Interpretation ^d
			B1 N = 122	B2–B3 N = 129			
Thickness of left ovary (mm)	246				<0.001	−0.59 (−0.68, −0.49)	Very large
N (non-missing)		246	120	126			
Median (Q1, Q3)		11.5 (8.8, 13.3)	9.5 (7.5, 11.6)	13.0 (11.2, 14.4)			
Volume of left ovary (cm ³)	246				<0.001	−0.62 (−0.70, −0.52)	Very large
N (non-missing)		246	120	126			
Median (Q1, Q3)		1.86 (1.05, 2.84)	1.26 (0.64, 1.83)	2.60 (1.85, 3.67)			
Follicles in visual field of left ovary (N)	234				<0.001	−0.38 (−0.50, −0.24)	Large
N (non-missing)		234	115	119			
Median (Q1, Q3)		5.00 (3.00, 6.00)	4.00 (3.00, 5.00)	5.00 (4.00, 8.00)			
The largest follicle in left ovary (mm)	236				<0.001	−0.47 (−0.57, −0.34)	Very large
N (non-missing)		236	115	121			
Median (Q1, Q3)		5.85 (4.40, 7.55)	5.00 (3.70, 6.50)	7.00 (5.50, 8.60)			
Length of right ovary (mm)	248				<0.001	−0.60 (−0.68, −0.50)	Very large
N (non-missing)		248	122	126			
Median (Q1, Q3)		25 (20, 31)	21 (17, 26)	29 (25, 33)			
Width of right ovary (mm)	248				<0.001	−0.52 (−0.62, −0.40)	Very large
N (non-missing)		248	122	126			
Median (Q1, Q3)		13.3 (10.5, 16.0)	11.1 (8.8, 13.9)	14.8 (12.5, 17.4)			
Thickness of right ovary (mm)	248				<0.001	−0.58 (−0.67, −0.48)	Very large
N (non-missing)		248	122	126			
Median (Q1, Q3)		11.7 (9.4, 14.2)	9.7 (8.2, 12.0)	13.6 (11.4, 15.2)			
Volume of right ovary (cm ³)	248				<0.001	−0.63 (−0.71, −0.53)	Very large
N (non-missing)		248	122	126			
Median (Q1, Q3)		2.10 (1.14, 3.41)	1.28 (0.69, 2.17)	3.05 (2.04, 4.26)			
Follicles in visual field of right ovary (N)	238				<0.001	−0.35 (−0.47, −0.22)	Large
N (non-missing)		238	118	120			
Median (Q1, Q3)		5.00 (4.00, 7.00)	4.00 (3.00, 6.00)	5.50 (4.00, 8.00)			
Breast ultrasound parameters							
Left breast thickness at the nipple (mm)	250				<0.001	−0.97 (−0.98, −0.96)	Very large

TABLE 2 (Continued)

Characteristic	N	Tanner breast			p-value ^b	Effect size (ES) (95% CI) ^c	Interpretation ^d
		Overall N = 251 ^a	B1 N = 122	B2–B3 N = 129			
N (non-missing)		250	121	129			
Median (Q1, Q3)		8 (4, 16)	4 (3, 5)	16 (11, 21)			
Right breast thickness at the nipple (mm)	250				<0.001	−0.97 (−0.97, −0.95)	Very large
N (non-missing)		250	121	129			
Median (Q1, Q3)		9 (4, 16)	4 (3, 5)	16 (12, 21)			

^aData are presented as median (Q1–Q3) or n (%), as appropriate.

^bGroup comparisons were performed using the Kruskal–Wallis test, Pearson's chi-squared test, or Fisher's exact test, as appropriate.

^cEffect size (ES) was expressed as rank-biserial correlation coefficient for continuous variables and Cramer's V for categorical variables.

^dTanner breast stages B2 and B3 were combined for comparative analyses.

^eRepresents the number of participants with available measurements (n).

in multivariable analysis. Ovarian indicators also contributed meaningfully: higher left ovarian follicle count and increased right ovarian length were associated with more advanced breast stages, whereas a greater number of right ovarian follicles was inversely associated with Tanner B2–B3 in the multivariable model.

Overall, these findings demonstrate that pelvic ultrasound reflects early pubertal development. It even showed stronger associations than anthropometric indicators. The strong correlation between thelarche and internal genital development supports the concept that external pubertal signs are closely associated with internal reproductive maturation. This highlights pelvic ultrasonography as a valuable adjunct to Tanner staging, particularly in cases where visual assessment is uncertain or complicated by increased adiposity. Taken together, our results underscore the importance of combining external anthropometric and internal ultrasonographic markers to improve the accuracy of pubertal assessment in clinical and research settings.

Although Tanner staging has long been the standard clinical method for assessing pubertal development, it relies on subjective visual inspection and palpation. With rising rates of childhood obesity, distinguishing glandular from adipose tissue has become increasingly challenging, often resulting in misclassification. This limitation was highlighted in the study by Carlson et al.,¹⁶ which demonstrated discordance between Tanner breast staging and true glandular breast morphology, particularly among overweight and obese girls. These findings emphasize the need for complementary objective tools to evaluate key pubertal milestones such as thelarche and menarche.

Anthropometric measurements have traditionally been used to evaluate nutritional status and adiposity, with BMI, waist circumference, waist-to-hip ratio, waist-to-height ratio, and skinfold thickness being among the most widely applied markers.¹⁷ Consistent with prior findings by Biro et al.¹⁸ and Rosenfield et al.,¹⁹ we observed that girls who had reached Tanner B2–B3 exhibited higher skinfold thickness and greater body circumferences compared with those in Tanner B1. Importantly, our results extend earlier observations by

demonstrating that body circumferences—particularly thigh, chest, and waist—show very large effect sizes and stronger associations with Tanner staging than skinfold thickness, suggesting that somatic growth and overall body proportions may more precisely reflect pubertal progression than subcutaneous fat distribution alone.

Because IPT may mimic early activation of the hypothalamic–pituitary–gonadal (HPG) axis, differentiating IPT from central precocious puberty (CPP) remains essential. Close follow-up every 3–6 months is recommended because CPP may lead to accelerated bone maturation, reduced adult height, and psychosocial difficulties.^{20,21} Pelvic ultrasound findings indicative of advanced ovarian and uterine development can support identification of premature HPG axis activation. In line with the observations of Cantas-Orsdemir et al.,²¹ our study demonstrated markedly greater uterine and ovarian measurements in girls classified as Tanner B2–B3, supporting the value of pelvic ultrasound as an adjunctive diagnostic tool.

Previous research has identified uterine length as one of the most reliable ultrasound parameters for distinguishing CPP from IPT, with Badouraki et al.²² and Wen et al.²³ reporting cut-off values between 2.45 and 3.83 cm depending on age. The median uterine body length in our Tanner B2–B3 group (2.35 cm) closely aligns with these reported thresholds. Additional estrogen-dependent morphological changes—such as fundal enlargement and a fundus-to-cervix ratio exceeding 1—were also evident in our sample (mean ratio 1.37 ± 0.77), consistent with previous findings.^{24–29} Although uterine volume was not directly assessed, our observation that uterine body length and thickness were strong predictors of Tanner B2–B3 (OR = 1.12 and OR = 1.23, respectively) supports the concept that uterine morphometry reflects internal genital maturation during early puberty.

Ovarian maturation, driven by increasing follicle-stimulating hormone (FSH) secretion, represents another key indicator of early pubertal onset. Prior to puberty, the ovaries are typically located in the upper part of the pelvis and are mainly composed of stroma with small follicular formations. In our cohort, the right ovary appeared to be more easily visualized and can be measured with greater accuracy

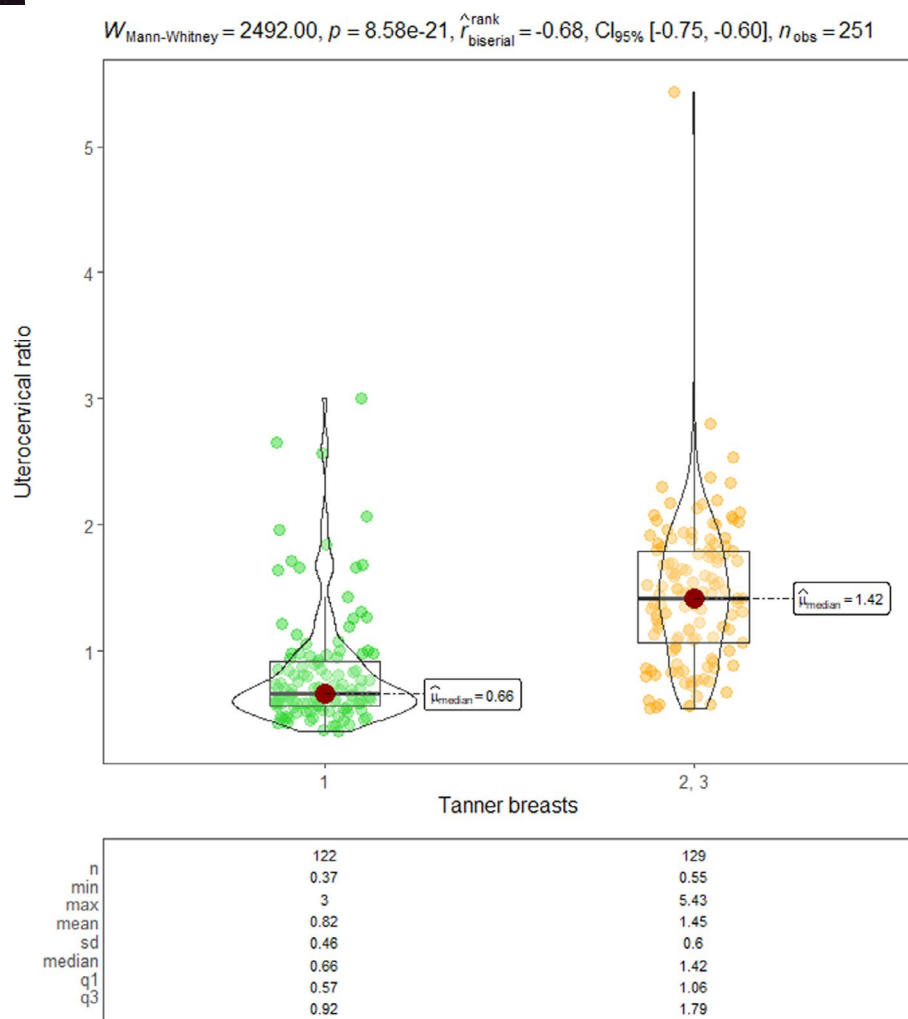


FIGURE 3 Uterocervical ratio according to Tanner breast stage groups. Violin plots with embedded boxplots demonstrate the distribution of uterocervical ratio values in girls classified as Tanner breast stage B1 vs. combined Tanner stages B2–B3. Red dots indicate group medians. The Mann–Whitney *U* test demonstrated a significant difference between groups ($W = 1438.00$, $p < 0.001$), with a large effect size (rank-biserial correlation = 0.82).

during a transabdominal ultrasound. We found a positive association between the length of the right ovary and Tanner stages B2 to B3, with an odds ratio of 1.11 (95% CI 1.02–1.21, $p=0.013$).

Volumetric growth during puberty is closely linked to the increase in both the number and size of ovarian follicles, showing even greater variability than growth in the uterus. Up to Tanner Stage B3, the presence of small and medium-sized follicles is predominant. A higher number of right ovarian follicles was inversely associated with Tanner B2–B3 in the multivariable model ($OR=0.72, p=0.036$), whereas left ovarian follicle count showed a positive association ($OR=1.39, p=0.035$). Notably, both variables demonstrated positive associations with Tanner stage in univariable analysis. The divergence in direction observed in the multivariable model likely reflects multicollinearity between bilateral ovarian parameters rather than a biologically meaningful asymmetry. During model optimization, similar reversals of coefficient direction were observed for other correlated ovarian variables, supporting the possibility of coefficient instability in the presence of highly interrelated predictors. This interpretation

is further supported by the correlation matrix presented in Figure S3, which demonstrates substantial correlations among several ovarian ultrasound variables, consistent with multicollinearity between these predictors. Given the physiological symmetry of ovarian maturation, a biologically meaningful opposite effect is unlikely. Therefore, these findings should be interpreted cautiously and should not be considered evidence of differential ovarian function.

Consistent with Wen et al.²³ and Bertoncello et al.,³⁰ we found that ovarian volume increased significantly from Tanner B1 to Tanner B2–B3. The strong association between Tanner stage and ovarian parameters in our study further reinforces the sensitivity of ovarian growth to early hormonal stimulation, supporting their diagnostic usefulness in differentiating variants of early puberty.

Eckert-Lind et al. reported a secular trend toward earlier pubertal onset, demonstrating that the age at thelarche decreased by nearly 3 months per decade between 1977 and 2013.⁵ In the present study, the estimated midpoint of transition to Tanner breast stage 2 (B2) occurred at 10.7 years of age. The probability of attaining

TABLE 3 Logistic regression analysis of ultrasound predictors associated with Tanner breast stages B2–B3 vs. B1.

Characteristic		Tanner breasts		OR (univariable)	OR (multivariable)
		B1 (N = 101)	B2–B3 (N = 111)		
Length of uterine body (mm)	Mean ± SD	13.4 ± 5.7	28.0 ± 10.5	1.23 (1.16–1.30, <i>p</i> < 0.001)	1.12 (1.04–1.21, <i>p</i> = 0.004)
Uterine thickness (mm)	Mean ± SD	7.6 ± 3.3	15.7 ± 6.1	1.45 (1.31–1.60, <i>p</i> < 0.001)	1.23 (1.07–1.40, <i>p</i> = 0.003)
Follicles in visual field of left ovary (N)	Mean ± SD	4.5 ± 2.0	6.2 ± 3.2	1.29 (1.14–1.45, <i>p</i> < 0.001)	1.39 (1.02–1.89, <i>p</i> = 0.035)
Length of right ovary (mm)	Mean ± SD	21.9 ± 6.0	29.4 ± 6.5	1.21 (1.14–1.28, <i>p</i> < 0.001)	1.11 (1.02–1.21, <i>p</i> = 0.013)
Follicles in visual field of right ovary (N)	Mean ± SD	4.7 ± 2.1	6.2 ± 3.0	1.25 (1.12–1.41, <i>p</i> < 0.001)	0.72 (0.53–0.98, <i>p</i> = 0.036)

Note: Data are presented as mean ± SD. Variables included in the final multivariable model were selected according to model fit statistics (AIC, BIC) and clinical interpretability. The dependent outcome variable in the logistic regression model was Tanner breast stage (B2–B3 vs. B1).

Abbreviations: CI, confidence interval; OR, odds ratio.

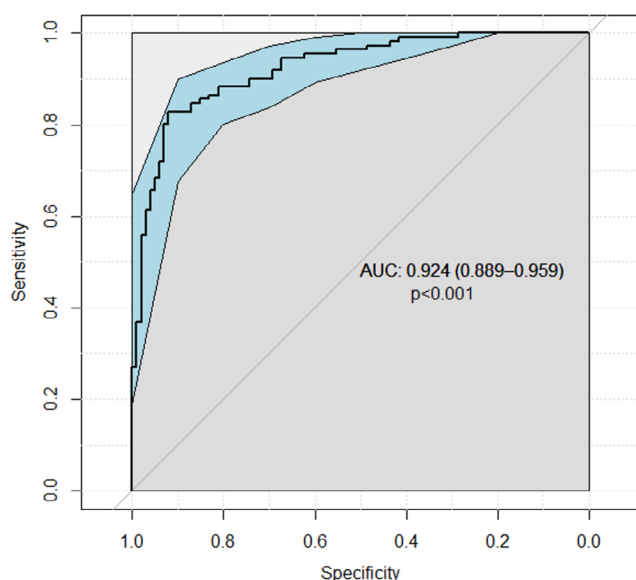


FIGURE 4 ROC curve of the logistic regression model distinguishing Tanner breast stages B2–B3 from B1. The logistic regression model demonstrated excellent discriminatory performance for identifying girls in Tanner breast stages B2–B3 vs. B1, with an area under the curve (AUC) of 0.924 (95% CI 0.889–0.959, *p* < 0.001). The shaded area represents the 95% confidence interval.

Tanner stage B2 increased after 10 years of age, reached its peak between 11 and 12 years, and subsequently declined. These findings are consistent with those reported by Eckert-Lind et al., who also highlighted substantial geographical variation in the timing of pubertal onset, with the earliest onset observed in the United States

(8.8–10.3 years) and the latest in Africa (10.1–13.2 years). Similarly, Biro et al. demonstrated racial and ethnic differences in pubertal timing, reporting median ages at breast stage 2 onset of 8.8, 9.3, 9.7, and 9.7 years among African American, Hispanic, non-Hispanic White, and Asian girls, respectively.³¹ Despite these findings, data on the age at thelarche onset in European populations remain limited, underscoring the need for further research in this region.

The primary strength of this study is its cross-sectional design and the comprehensive assessment of anthropometric and ultrasound parameters in a relatively large cohort of healthy premenarcheal girls. All clinical assessments were conducted by two experienced and highly qualified physicians, ensuring consistency of ultrasound examinations.

However, several limitations should be acknowledged. Although the overall sample size was adequate for a clinical ultrasound study, considerations related to statistical power in the multivariable model remain important. The number of events per variable (EPV) was approximately 22, which is within generally accepted thresholds for logistic regression models. However, the relatively wide confidence intervals observed for some predictors—particularly ovarian follicle counts—suggest limited precision of the estimates, and the results should therefore be interpreted with caution.

Additionally, we did not perform invasive assessments, such as serum hormone measurements. However, we prioritized participant safety by avoiding unnecessary procedures that may involve discomfort or potential risks, which was particularly important given the young age of the study population.

Although transabdominal ultrasound is the standard imaging approach in pediatric populations, visualization of small ovarian follicles may still be affected by technical factors such as bowel gas and individual differences in body habitus.

Another limitation of this study is the absence of blinding of the sonographers to the clinical Tanner stage, which may have introduced observer bias and potentially contributed to the high diagnostic performance (AUC) of the model. Nevertheless, previous studies in which ultrasound staging was assessed using independent observers have also demonstrated good reliability and reproducibility of breast ultrasound assessment, supporting the robustness of ultrasound-based pubertal staging. Future studies incorporating blinded assessment will be important to further validate the diagnostic performance of predictive models.

5 | CONCLUSION

A novel and clinically meaningful finding is the close correlation between thelarche and internal genital maturation on pelvic ultrasound, paralleling Tanner breast stages and supporting the concept of correlated external and internal pubertal development.

Ultrasound parameters exhibited even stronger associations with Tanner staging than anthropometric markers. Uterine body length and uterine thickness were markedly increased in Tanner B2–B3 and remained strong independent predictors in multivariable analysis (uterine body length: OR=1.12, 95% CI 1.04–1.21, $p=0.004$; uterine thickness: OR=1.23, 95% CI 1.07–1.40, $p=0.003$). Ovarian characteristics contributed meaningfully as well: a higher follicle count in the left ovary (OR=1.39, 95% CI 1.02–1.89, $p=0.035$) and greater right ovarian length (OR=1.11, 95% CI 1.02–1.21, $p=0.013$) were positively associated with B2–B3, whereas a higher number of right ovarian follicles reduced the odds of being in this stage (OR=0.72, 95% CI 0.53–0.98, $p=0.036$). The logistic regression model distinguishing Tanner B2–B3 from B1 demonstrated excellent discrimination (AUC=0.924, 95% CI 0.889–0.959), underscoring the strong predictive value of pelvic ultrasound in early pubertal assessment.

Although our findings demonstrate strong associations between Tanner breast staging and pelvic ultrasound parameters, pubertal stage in this study was defined using clinical and ultrasonographic criteria and should not be interpreted as direct evidence of hypothalamic–pituitary–ovarian axis activation.

These results support the integration of pelvic ultrasound with targeted anthropometry to improve the objective assessment of pubertal stage, particularly when clinical inspection is uncertain or complicated due to differences in body habitus. Future studies with hormonal profiling and larger samples should refine cut-offs and validate implementation in routine care.

AUTHOR CONTRIBUTIONS

Conceptualization and design: AC, ZSB, ZB, and JT. **Methodology:** ZB and JT. **Software:** AC. **Validation:** AC and ZB. **Formal analysis:** AC, ZSB and EJ. **Investigation:** AC and RM. **Resources:** AC. **Data curation:** ZB and JT. **Writing—original draft preparation:** ZSB, AC, and EJ. **Writing—review and editing:** ZSB, AC, ZB, JT, and DR. **Visualization:** EJ. **Supervision:** ZB and JT. **Project administration:** AC, ZB, JT, and DR. **Funding acquisition:** N/A. Andrius Cekuolis and Zivile Sabonyte-Balsaitiene contributed

equally to this work and share first authorship. All the authors have read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The study was approved by the Vilnius Regional Biomedical Research Ethics Committee (Approval No. 2023/5-1402-969, approved on May 9, 2023). All participants and their parents or legal guardians received detailed information about the study. Written informed consent was obtained from parents or legal guardians, and written assent was obtained from all participating girls before enrollment. All procedures were performed in accordance with the ethical standards of the institutional and national research committee and with the Declaration of Helsinki and its later amendments.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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