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Inhibin B and luteinizing hormone levels in girls aged 6-11 years from NHANES III, 1988-1994

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Abstract

Objective—Evaluate inhibin B and luteinizing hormone (LH) levels in a large, representative cross-sectional sample of U.S. girls and characterize the relationships of these laboratory values with age, clinical signs of puberty, and other correlates.

Design—Cross-sectional analysis of LH and inhibin B in banked serum from 720 girls aged 6-11 years who participated in the Third National Health and Nutrition Examination Survey (NHANES III).

Measurements—Levels of inhibin B and LH, race, ethnicity, and anthropometric measurements were compared for all girls. Visual assessment of pubertal stage was performed on girls 8 years and older. A two-part model was used to establish normative data and tobit regression models were used to evaluate associations with participant characteristics. Receiver operating characteristic (ROC) analysis was performed to identify optimum cut points predictive of puberty onset.

Results—Mean hormone levels progressively increased with age. LH levels progressively increased with pubertal stage. Inhibin B levels increased gradually from breast stage I to II, then more sharply to peak at stage III, followed by a plateau at stages IV and V. ROC curves indicated both hormones were consistent with pubertal onset as indicated by breast stage II.

Conclusions—This study characterizes inhibin B and LH values in a large, representative cross-sectional sample of U.S. girls. Inhibin B can be a useful tool in combination with other clinical and biochemical parameters to evaluate gonadal function as a reflection of pubertal progression in girls.

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Keywords

Inhibin B; luteinizing hormone; NHANES; puberty; girls

Introduction

The importance of inhibins in pubertal development has only relatively recently begun to be characterized, sparked by the development of enzyme-linked immunosorbent inhibin assays that distinguish between inhibins A and B.¹ Inhibin B is a heterodimeric glycoprotein consisting of an alpha subunit linked to a beta-B subunit.² It is secreted by granulosa cells in small antral follicles, and plays an important role in the follicle stimulating hormone (FSH) feedback loop.³⁻⁵

Previous reports have characterized inhibin B levels in girls at different stages of childhood. During the period in infancy of increased hypothalamic-pituitary-gonadal activation, inhibin B levels are elevated, signifying importance in functional follicle maturation. Levels then fall so that during the prepubertal period, they are much lower and often unmeasurable.⁶ Evaluation in school-age girls who are clinically prepubertal has revealed a progressive increase in inhibin B levels with age, reflecting increasing follicular activity.⁷ This finding is consistent with reports of ovarian ultrasonography in girls aged 7-10 years showing an increase in follicle development that can precede physical changes of puberty.⁸ Evaluation during puberty has shown a slow increase from Tanner breast stage I (B1) to B2, with an increased rate of rise to B3 and variable increases from B4-B5.^{7,9-11} After the onset of menses, inhibin B rises during the follicular phase, reaching its peak shortly after the LH peak, and then decreases during the luteal phase, reflecting secretion by antral follicles.³

Serum luteinizing hormone (LH) and FSH concentrations in relation to puberty among girls have been known for some time to have a profile of mean levels gradually increasing from slightly before the physical onset of puberty (B2) until menarche.^{12,13} More recent profiles are similar, albeit with lower values using more recent gonadotropin assays.¹⁴ The relationship of gonadotropins to inhibin B has been recently studied suggesting a rise of both throughout puberty, or in a report of a limited number of girls, with a positive correlation with LH before and during early puberty.^{7,10}

The Third National Health and Nutrition Examination Survey (NHANES III), conducted from 1988-1994, involved interviews, physical examination, and collection and banking of serum from U.S. children.¹⁵ This cross-sectional sample provides a unique opportunity to evaluate data from a large, nationally representative sample of U.S. boys and girls. In 2007, serum specimens were utilized for assays of inhibin B and LH levels with the original intents of characterizing the relationship between these hormones, with previously reported serum lead levels, age, and the clinical onset of puberty.¹⁶ Given limitations in the quantity of stored sera, it was determined that the most useful hormones to study would be inhibin B and LH. These were selected based on information present or lacking in the existing literature, the relatively increased rise of mean levels of LH among girls with puberty compared to FSH, and the potential applicability of further data. The objectives of our study were to examine the LH and inhibin B levels in girls, including relationships with age and pubertal staging, thereby generating normative data, as well as to systematically evaluate associations with other participant characteristics.

Materials and Methods

Approval for this study was obtained by the Centers for Disease Control and Prevention National Center for Health Statistics Research Ethics Review Board. Our target population consisted of 1589 girls who participated in NHANES III from 1988-1994, and were aged 6.0- 11.99 years at the time of an initial household interview. Informed consent was obtained before participation in the survey. Initial sampling was based on a complex, multistage clustered probability sampling design that oversampled non-Hispanic blacks and Mexican-Americans to provide more precise estimates for these groups. Data were subsequently weighted to represent the entire U.S. population of girls for the included ages from 1988-1994. Reweighted sample weights were obtained from the initial sample weights and those calculated for the analytic subsample of girls who had stored residual sera available using propensity score weights. All statistical estimates were adjusted for these complex survey design effects namely, strata, probability sampling units (PSU), and the new reweighted sample weights. Racial/ethnic classifications utilized by NHANES III were also used to characterize our population. Further NHANES III design details are publicly available in the Plan and Operations outline.¹⁵

A total of 705 girls with available banked serum were included in this study. The remaining girls in the original cohort did not have adequate residual serum available for testing. Serum was obtained by venipuncture at the time of clinical exam and categorized as morning, afternoon, or evening based on the timing of the blood draw. All subjects had a physical exam by a participating pediatrician and anthropometric assessments. Visual assessment for breast and pubic hair development was performed without palpation of breast tissue in girls aged 8.0-11.9 years. Development was characterized according to apparent Tanner Stage. Girls 8 years or older were asked about menarcheal status.

Blood samples were obtained from the National Center for Health Statistics/Centers for Disease Control and Prevention. Hormone assays were performed by Rules-Based Medicine, Inc. (<http://www.rulesbasedmedicine.com>). LH levels were measured using the LH ELISA kit (Bio-Quant BQ049F; Bio-Quant, Inc., San Diego, CA, USA), a solid-phase direct sandwich method. Limit of detection (LOD) for LH was 0.05 mIU/ml. Intra-assay precision ranged from 6.18-10.58 % and inter-assay precision ranged from 8.13 to 11.57%. Inhibin B was measured using the DSL-10-84100 ACTIVE ® Inhibin B ELISA assay (Diagnostic Systems Laboratories, Inc., Webster, TX, USA, Gen I assay), an enzymatically amplified two-site two-step sandwich-type immunoassay. LOD for inhibin B was 7.0 pg/ml. A substitution of LOD/ 2 was used in the calculation of descriptive statistics for values that were below the LOD, and thus not accessible to the authors. Intra-assay precision ranged from 3.5-5.6 % and inter-assay precision ranged from 6.2-7.6%. Extreme or potentially outlying values for hormones were reviewed in light of other available data for individuals, and judgments were made concerning inclusion based on clinical feasibility.

Other covariates were obtained from the NHANES III database, including race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican-American, other), census region (Northeast, Midwest, South, West), poverty-income ratio (PIR), health rating, cigarette smoke exposure, and presence of anemia and iron deficiency. PIR was defined as the total household income divided by the poverty threshold for the year of the interview, with higher values indicating increased household income. Iron deficiency was defined as 2 out of 3 measures below age-specific cutoff values for transferrin saturation, serum ferritin, and erythrocyte protoporphyrin, while anemia was defined as a hemoglobin value the 5th percentile for age.^{17,18} Health ratings were parental reports of girls' health status ranging from 1 (excellent) to 5 (poor). Preliminary statistical analysis revealed no significant

differences in results based on timing of blood draw so data were pooled for subsequent evaluation.

Several anthropometric indices were derived. Body mass index (BMI) was calculated as weight in kilograms/ height in meters squared. Triceps and subscapular skinfold thickness were measured in millimeters. A two compartment body composition model was built using subcutaneous fatness (from sum of triceps + subscapular skinfolds) to predict body weight (consisting of fatness and lean mass) in regression models. The residuals/error terms of the regression models were defined as a measure of lean mass for each girl in subsequent multivariate analyses.

Statistical Methods

Descriptive statistics for continuous variables were presented as means and standard errors (SE) or medians and standard errors (SE), where appropriate, and categorical variables as percentages and confidence intervals (95% CI) using complex survey procedures. We used non-parametric graphical exploratory data analysis (EDA) ¹⁹ techniques to study monotonic trends in hormone levels across age. Subsequently, a penalized beta-spline smoother was used to present the pattern of hormone changes with age.²⁰ This smoother is resistant to outlying values and is able to capture any patterns of hormone levels associated with normal growth.

Smoothed percentiles and Z-scores for normative LH and inhibin B hormone values were calculated for ages 6.00 to 11.99 years in 6-month intervals. A 2-part-approach of percentile estimation was used to accommodate the high proportion of LOD values for LH and inhibin B. For part one, we modeled the continuous part of the distribution above the LOD using LMS method which summarizes hormone measurements using penalized maximum likelihood and cubic splines with 3 smoothed curves L M S.²¹ They denote: the Box-Cox power transformation (L) term for normalizing the measured covariate, the median (M) and the generalized coefficient of variation (S). The second part involved modeling for the estimated proportion below LOD. And then using a closed-form formula, we calculated the smoothed percentile estimates. All model assumptions were met. We used the worm plots and goodness-of-fit statistics for logistic regressions for continuous and discrete parts of the distribution respectively.²² A full description of the methods for this 2-part-approach has been described elsewhere.²³ Additionally, unsmoothed percentiles for the hormone levels were estimated using standard non-parametric ranking methods separately within breast and pubic hair stage categories.

Tobit regression models were used to study associations between hormone and participant descriptors. The use of tobit models facilitates efficient use of all parts of the distribution by applying maximum likelihood regression techniques to model both continuous and discrete parts of the data.²⁴ The use of the tobit regression models thus enabled us to obtain valid associations while accommodating the truncated distributions of the outcome variables, viz., LH and inhibin B, due to values below the LOD. Hormone concentrations were re-expressed to approximate normality to meet regression model assumptions. Consequently, regression estimates are presented in log-natural units as well as in back-transformed units. This approach has been used successfully elsewhere for clinical laboratory data.^{25,26}

Receiver operating characteristic (ROC) curves were used to select optimal hormone cutoffs for correctly identifying those girls with B2 compared with those with B1. These analyses did not include girls with B3 or B4. Because our outcome of interest is not a disease condition, we used minimal misclassification as the criterion for selecting optimal cut-offs, rather than favoring either sensitivity or specificity. To evaluate the performance of the

hormone cut-offs in the study population, we used cross-classification tables to assess the diagnostic performance of observed B2 vs. the new optimal cut offs.

All statistical analyses were carried out using PC SAS 9.2 (SAS, Cary, NC, USA). Statistical significance was declared at a 2-tailed p-value of <0.05.

Results

LH levels were completed for 705 girls, with 689 having inhibin B results. Table 1 characterizes sample demographics, with percentages weighted to represent the racial/ethnic makeup of the U.S. population. Mean serum levels tended to increase with age for LH and inhibin B, with the exception of a decrease in mean LH values at age 9 years. Median values for both LH and inhibin B only surpassed the LOD at 10 years of age and older, as the younger age groups had >50% of girls with levels below the limit of detection. Eight outlying LH values were identified; 6 of these in girls < 8 years of age, all with values higher than expected. All outliers were included in the statistical analysis. Only 3 potentially outlying Inhibin B values were identified, all with elevated levels, but with only one girl <8 years of age. Two of the elevated values were in fully pubertal girls. All three values were included in the analysis. Figures 1a and 1b graphically depict the distribution of levels of LH and inhibin B, respectively, based on participant age. When values were smoothed, LH gradually increased from age 8-10 years, with a steeper rate of increase beginning around 10 years of age. Conversely, inhibin B levels showed a less rapid but consistent increase throughout the ages measured.

Visual assessment of breast development, with assignment to apparent breast stage, was performed in 417 girls, while 411 had Tanner staging for pubic hair available. Thirty-five girls reported being post-menarcheal, with a mean reported age at menarche of 11.48 ± 0.04 years. LH values increased with progression of breast staging and pubic hair staging. Inhibin B values increased with breast and pubic hair staging through stage III, but plateaued at stage IV.

LH and inhibin B values were below the level of detection in 64.9 % and 62.0 % of girls characterized as B1. The percentage of girls below the level of detection for LH decreased with progression through breast staging, and all girls who were B4 or greater had detectable LH levels. Concordant with the mean values, the percentage of participants with detectable inhibin B increased as girls advanced to B3 staging. However, the percentage of undetectable values increased in those who had B4 or greater staging or were post-menarcheal.

Figures 2a and 2b graphically depict the distribution of LH and inhibin B levels in relation to age and breast stage. While illustrating the trends described above, the figures also highlight the inter-individual variation in stages within each age and hormone level. This was especially true for inhibin B levels in girls with B4 or greater staging.

One aim was to establish normative data for this population based on age and pubertal stage using the two-part model to correct for large percentages of values below the LOD. Results for LH (Table 2) and inhibin B (Table 3) are presented in the form of smoothed percentiles and Z-scores for age, and unsmoothed percentiles for pubertal stage. We would like to emphasize that the coefficients of variation presented in the smoothed percentile tables (2 and 3) only applies to the data above the LOD. As a result, it is not very useful for the ages they represent – even though they provide a partial sense of precision around the point estimates.

Our ROC analysis indicated that both hormones predicted onset of puberty as measured by B2 breast development, with area under the curve substantially greater than one half. The optimal LH cut-point was found to be 1.04 mIU/ml, (95% confidence interval (CI): 0.71-1.37). This cut-point yielded 77.56% agreement with a 9.24% false negative rate and a 13.20% false positive rate. The optimal cut-point identified for inhibin B was 17.89 pg/ml (95% CI: 11.63- 24.15) with an agreement of 76.57%, a false negative rate of 12.54% and a false positive rate of 10.89%.

Tobit regression models used to evaluate associations of hormone levels with age, race, health rating, anemia-status, PIR, census, region, body mass index, lean mass, skin-fold measurements, and clinical pubertal status/breast staging revealed a significant association between age (in years) and LH levels (Tobit coefficient β ($\pm$ SE): 0.29 ± 0.11 log natural (Ln)-mIU/ml/year, $p = 0.0100$; back transformed estimates: 1.34, 95 % CI: 1.07 – 1.67 mIU/ml/year) when controlled for the other characteristics. The same model showed that girls from the Midwest tended to have higher LH levels (β ($\pm$ SE): 1.02 ± 0.30 Ln-mIU/ml, $p = 0.006$; back transformed estimates: 2.79, 95 % CI: 1.55 – 5.00 mIU/ml higher on the average) compared with all other regions. Girls classified as non-Hispanic white had a tendency toward lower LH levels (β ($\pm$ SE): -0.91 ± 0.32 Ln-mIU/ml, $p = 0.004$; back transformed estimates: 0.40, 95%CI: 0.22 – 0.75 mIU/ml lower on the average) compared with all other racial groups. No independent relationships were seen between LH levels and PIR, other census regions, skinfold measurements, or lean mass when controlled for the other variables.

Evaluation of associations of inhibin B with the same panel of characteristics revealed a significant association with age in years (β ($\pm$ SE): 0.40 ± 0.08 Ln- pg/ml/year, $p < 0.0001$; back transformed estimates: 1.50, 95 % CI: 1.28 – 1.76 pg/ml/year) when controlled for the other variables. Sum of skinfolds (mm) had a negative association with inhibin B level (β ($\pm$ SE): -0.03 ± 0.007 Ln- pg/ml per mm, $p < 0.000$; back transformed estimates: 0.97, 95% CI: 0.96 – 0.98 pg/ml/ lower on the average per 1 mm increase in skinfolds). No other variables were found to have significant independent relationships.

Discussion

Relative patterns and frequencies for stages of breast and pubic hair development were consistent with expectations for this age range. This study provides a characterization of inhibin B and LH levels in this large, representative population of U.S. girls. Our findings of a progressive LH increase with age and pubertal stage are consistent with previous reports exploring these relationships.^{14,27} Previous reports regarding inhibin B levels in relation to age and pubertal staging in other populations have been mostly consistent with our findings, showing the positive association with age, as well as the progressive increase within B1, then to B2, followed by a sharper rate of rise with a peak before menarche.^{6,7,9-11,27} These findings are consistent with a progressive increase in follicular development and recruitment that culminates with a peak in follicular activity during B3, before the initiation of menarche and the subsequent decrease in inhibin B levels seen during the luteal phase of the menstrual cycle.⁹ Changes in inhibin B levels by staging for pubic hair development mirrored those seen for breast development. This pattern likely reflects progression of central puberty.

ROC analysis yielded an agreement (efficiency) of approximately 77% for both LH and inhibin B as indicators of puberty onset, as measured by B2 development. Using these cutoffs approximately 23% of girls would be misidentified. Although this minimal misclassification rate is relatively high for either test to be useful in isolation for the identification of true puberty, in combination with a more complete clinical picture, each could prove beneficial. Additionally, visual inspection for breast staging (the outcome

measure for the ROC analysis) without palpation could lead to misclassification of a prepubertal girl in of itself, especially those with high body fat, as higher BMI has been found to be associated with higher breast area.²⁸ It therefore seems unlikely that these new hormone cut-offs for predicting onset of puberty are underperforming in a nationally diverse and representative sample of girls like those of the current study. Inhibin B can also be useful as a marker of gonadal function in girls with hypothalamic-pituitary axis abnormalities who are at risk for primary gonadal dysfunction.²⁹ For these patients, representative normative data are crucial for appropriate medical decision making and counseling regarding fertility.

Other research has found varying results regarding fat mass and a positive association with pubertal onset and progression.³⁰⁻³³ This study did not find any significant association between hormones and body mass index when controlling statistically for other potential covariates. There was a negative association between skinfold measurements and inhibin B level, which has not, to our knowledge, been previously described. An association with LH and lean body mass was noted originally, but became non-significant once corrected for pubertal staging, and was most likely a reflection of progression through puberty, as has been previously noted.³⁰

The negative association of LH levels and non-Hispanic white race is consistent with previous reports.^{34,35} Our analysis also showed an independent positive association between LH and Midwest origin. This effect could be the result of a population difference with a greater portion of clinically prepubertal girls having the increase in LH levels which occurs shortly before the transition to B2. It could also reflect a higher LH level across all pubertal stages. Such trends could possibly be due to an environmental effect or to residual confounding. However, these associations must be interpreted cautiously as, although the variables were corrected for each other, there is a possibility that they interact with each other ways that are not obvious.

There are several limitations to our study. Tanner staging was not performed in girls less than 8 years old; however, some of these girls may have had clinical signs of puberty. Staging performed by visual inspection without palpation may have resulted in erroneous assignments. If this occurred, one would expect the described onset of breast stage 2 to be somewhat younger, especially in heavier girls. The immunoradiometric LH assay used in our study is not as sensitive as other immunochemiluminometric LH assays, which may be more useful in younger children who have hormone changes but no clinical signs of puberty.¹⁴ Since the serum samples from our study population were analyzed, a more sensitive second-generation inhibin B assay has become available.³⁶ Evaluation with this assay would have been preferable given the high percentage of prepubertal girls with values below the level of detection. Nevertheless, this study comprises the largest representative sample of U.S. girls in which both inhibin B and pubertal staging were evaluated.

In conclusion, this study provides normative data for inhibin B and LH levels in a large population of U.S. girls aged 6-11 years. Levels showed a clear increase with age as well as with progression through puberty to B3, with high inter-individual variation thereafter. LH levels also increased with age, with a steeper rate of increase after the onset of puberty. Both hormones can be useful instruments in adjunct with other clinical and biochemical markers in assessing onset of puberty.

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Figure 1a.

Scatter plot describing monotonic trends in smoothed LH values with age. Observed values are plotted as dots, while smoothed values are plotted as triangles. Data were statistically smoothed to generate a pattern of values that is resistant to outliers.

Figure 1b.

Scatter plot describing monotonic trends in smoothed inhibin B values with age. Observed values are plotted as stars, while smoothed values are plotted as squares. Data were statistically smoothed to generate a pattern of values that is resistant to outliers.

Figure 2a.
Three dimensional scatter plot of measured LH (mIU/ml) vs. age and breast stage.

Figure 2b.
Three dimensional scatter plot of measured inhibin B (pg/ml) vs. age and breast stage.

Table 1

Participant Characteristics

Descriptors	Categories	Count	Percent (95% CI)
Race	NH-W	146	65.3 (54.8 – 75.6)
	NH – B	258	17.6 (12.2 – 22.9)
	Mexican American	267	7.9 (5.1 – 10.6)
	Other	34	9.3(3.6 – 15.0)
Census Region	Northeast	61	17.2 (9.3 – 25.1)
	Midwest	153	27.4 (17.6 – 37.2)
	South	272	32.9 (20.9 – 44.9)
	West	219	22.5 (8.5 – 36.5)
Exam Session	Morning	271	30.7 (24.9 – 36.6)
	Afternoon	249	39.1 (34.2 – 44.1)
	Evening	185	30.1 (23.9 – 36.3)
Breast Stage (B)	B 1	191	53.8 (46.5 – 61.0)
	B 2	127	29.8 (21.4 – 38.1)
	B 3	61	10.6 (3.8 – 17.3)
	B 4+	38	5.9 (3.2 – 8.5)
Pubic Hair (PH)	PH 1	209	55.7 (47.5 – 64.0)
	PH 2	95	23.4 (16.5 – 30.4)
	PH 3	72	15.8 (5.4 – 26.3)
	PH4+	35	5.0 (2.3 – 7.7)
Menarcheal Status	Post-menarcheal	35	3.9 (1.8 – 6.0)
PIR	<1	301	53.1 (44.0 – 62.2)
	1-2	160	37.0 (27.0 – 47.0)
	2 +	50	9.9 (4.0 – 15.7)
Anemia and Iron Deficiency *	Anemic (count)	80	7.7 (4.7 – 10.6)
	Iron Deficient	32	2.3 (0.4 – 4.2)
Health Rating	Excellent	233	43.3 (35.1 – 51.6)
	Very Good	188	30.1 (24.1 – 36.2)
	Good	213	23.3 (18.4 – 28.2)
	Fair	69	3.1 (1.7 – 4.5)
	Poor	2	0.1 (0.0 – 0.3)

Abbreviations: NHW – Non Hispanic white; NHB- Non Hispanic black; PIR- Poverty Income Ratio.

* Iron deficiency was defined as 2 out of 3 measures below age-specific cutoff values for transferrin saturation, serum ferritin, and erythrocyte protoporphyrin according to the guidelines provided by the Centers for Disease Control and Prevention. Anemia was defined as hemoglobin value the 5th percentile for age.

Smoothed Percentiles for Luteinizing Hormone for Peri-pubertal US Girls Ages 6.00-11.99 (N=705) Using the 2-part Model

Table 2a

Age, y	Luteinizing hormone (mIU/ml)		Smoothed Percentiles				
	Smoothed Prob. of Exceeding LOD	Coefficient of Variation	10 th	25 th	50 th	75 th	90 th
6.00-6.49	0.09	1.43	<0.05	<0.05	<0.05	<0.05	<0.05
6.50-6.99	0.33	1.43	<0.05	<0.05	<0.05	0.12	0.51
7.00-7.49	0.41	1.43	<0.05	<0.05	<0.05	0.22	0.78
7.50-7.99	0.42	1.42	<0.05	<0.05	<0.05	0.31	1.06
8.00-8.49	0.42	1.42	<0.05	<0.05	<0.05	0.38	1.34
8.50-8.99	0.4	1.39	<0.05	<0.05	<0.05	0.37	1.32
9.00-9.49	0.38	1.34	<0.05	<0.05	<0.05	0.30	1.10
9.50-9.99	0.38	1.27	<0.05	<0.05	<0.05	0.32	1.08
10.00-10.49	0.44	1.21	<0.05	<0.05	<0.05	0.56	1.54
10.50-10.99	0.59	1.15	<0.05	<0.05	0.30	1.32	2.82
11.00-11.49	0.79	1.11	<0.05	0.16	1.07	2.56	4.59
11.50-11.99	0.90	1.08	<0.05	0.60	1.80	3.70	6.15

The coefficients of variation only apply to the data above the LOD.

Smoothed Z Scores for Luteinizing Hormone for Peri-pubertal US Girls Ages 6.00-11.99 (N=705) Using the 2-part-Model

Table 2b

Age, y	Smoothed Prob. of Exceeding LOD	Coefficient of Variation	Estimated Smoothed Z scores for Luteinizing hormone, mIU/ml						
			-1.5	-1	-0.5	0	0.5	1	1.5
6.00 -6.49	0.09	1.43	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.12
6.50 -6.99	0.33	1.43	<0.05	<0.05	<0.05	<0.05	0.06	0.28	0.77
7.00 -7.49	0.41	1.43	<0.05	<0.05	<0.05	<0.05	0.14	0.46	1.13
7.50 -7.99	0.42	1.42	<0.05	<0.05	<0.05	<0.05	0.19	0.63	1.53
8.00 - 8.49	0.42	1.42	<0.05	<0.05	<0.05	<0.05	0.23	0.80	1.92
8.50 - 8.99	0.4	1.39	<0.05	<0.05	<0.05	<0.05	0.21	0.78	1.88
9.00 - 9.49	0.38	1.34	<0.05	<0.05	<0.05	<0.05	0.17	0.66	1.55
9.50 - 9.99	0.38	1.27	<0.05	<0.05	<0.05	<0.05	0.18	0.66	1.49
10.00 - 10.49	0.44	1.21	<0.05	<0.05	<0.05	<0.05	0.37	1.02	2.05
10.50 - 10.99	0.59	1.15	<0.05	<0.05	<0.05	0.30	1.00	2.04	3.54
11.00 - 11.49	0.79	1.11	<0.05	<0.05	0.36	1.07	2.10	3.55	5.53
11.50 - 11.99	0.90	1.08	<0.05	0.22	0.85	1.80	3.13	4.92	7.22

The coefficients of variation only apply to the data above the LOD.

Weighted Unsmoothed Percentiles for Luteinizing Hormone by Pubertal Stages with Non-parametric Ranking for Peri-pubertal US Girls Ages 6.00-11.99(N=705)

Table 2c

Pubertal stage	Unweighted N	Proportion Exceeding LOD	10 th	25 th	50 th	75 th	90 th
B 1	191	0.35	<0.05	<0.05	<0.05	0.2	1.02
B 2	127	0.72	<0.05	<0.05	0.40	1.27	4.09
B 3	61	0.87	0.18	1.10	1.10	2.93	3.81
B 4+	38	1.00	0.19	1.92	3.02	6.33	11.65
PH 1	209	0.43	<0.05	0.04	0.04	0.2	0.67
PH 2	95	0.63	<0.05	0.04	0.36	2.34	4.35
PH 3	72	0.86	<0.05	0.35	1.1	2.91	4.23
PH 4+	35	1.00	0.19	1.59	2.68	6.1	11.65

Smoothed Percentiles for Inhibin B for Peri-Dubertal US Girls Ages 6.00-11.99 (N=705) Using the 2-Dart Model

Table 3a

Age, y	Inhibin B, pg/ml					
	Smoothed Prob. of Exceeding LOD	Coefficient of Variation	Smoothed Percentiles			
			10 th	25 th	50 th	75 th 90 th
6.00-6.49	0.09	0.39	<7.0	<7.0	<7.0	<7.0 <7.0
6.50-6.99	0.09	0.44	<7.0	<7.0	<7.0	<7.0 <7.0
7.00-7.49	0.10	0.50	<7.0	<7.0	<7.0	<7.0 <7.0
7.50-7.99	0.13	0.55	<7.0	<7.0	<7.0	<7.0 7.78
8.00-8.49	0.18	0.60	<7.0	<7.0	<7.0	<7.0 11.65
8.50-8.99	0.25	0.63	<7.0	<7.0	<7.0	<7.0 17.29
9.00-9.49	0.33	0.64	<7.0	<7.0	<7.0	11.31 24.21
9.50-9.99	0.43	0.66	<7.0	<7.0	<7.0	16.67 31.85
10.00-10.49	0.52	0.67	<7.0	<7.0	7.64	22.60 40.13
10.50-10.99	0.59	0.69	<7.0	<7.0	12.47	28.47 48.39
11.00-11.49	0.63	0.71	<7.0	<7.0	15.81	34.22 56.71
11.50-11.99	0.67	0.73	<7.0	<7.0	19.51	40.58 65.87

The coefficients of variation only apply to the data above the LOD.

Table 3b
Smoothed Z Scores for Inhibin B for Peri-pubertal US Girls Ages 6.00-11.99 (N=705) Using the 2-part Model

Age, y	Smoothed Prob. of Exceeding LOD	Coefficient of Variation	Estimated Smoothed Z scores for Inhibin B, pg/ml						
			-1.5	-1	-0.5	0	0.5	1	1.5
6.00 - 6.49	0.09	0.39	<7.0	<7.0	<7.0	<7.0	<7.0	<7.0	6.32
6.50 - 6.99	0.09	0.44	<7.0	<7.0	<7.0	<7.0	<7.0	<7.0	6.89
7.00 - 7.49	0.10	0.50	<7.0	<7.0	<7.0	<7.0	<7.0	<7.0	8.12
7.50 - 7.99	0.13	0.55	<7.0	<7.0	<7.0	<7.0	<7.0	<7.0	10.83
8.00 - 8.49	0.18	0.60	<7.0	<7.0	<7.0	<7.0	<7.0	7.15	15.61
8.50 - 8.99	0.25	0.63	<7.0	<7.0	<7.0	<7.0	<7.0	11.94	22.77
9.00 - 9.49	0.33	0.64	<7.0	<7.0	<7.0	<7.0	7.72	17.49	31.11
9.50 - 9.99	0.43	0.66	<7.0	<7.0	<7.0	<7.0	13.28	23.87	39.75
10.00 - 10.49	0.52	0.67	<7.0	<7.0	<7.0	7.64	18.74	31.01	48.80
10.50 - 10.99	0.59	0.69	<7.0	<7.0	<7.0	12.47	23.98	38.18	57.79
11.00 - 11.49	0.63	0.71	<7.0	<7.0	<7.0	15.81	29.01	45.32	66.92
11.50 - 11.99	0.67	0.73	<7.0	<7.0	<7.0	19.51	34.60	53.18	77.06

The coefficients of variation only apply to the data above the LOD.

Weighted Unsmoothed Percentiles for Inhibin B by Pubertal Stages with Non-parametric Ranking for Peri-pubertal US Girls Ages 6.00-11.99(N=705)

Table 3c

Pubertal Stage	Unweighted N	Proportion Exceeding LOD	10 th	25 th	50 th	75 th	90 th
B 1	187	0.38	<7.0	<7.0	<7.0	20.6	33.2
B 2	123	0.60	<7.0	<7.0	14.2	29.4	67.95
B 3	61	0.74	<7.0	14.85	40.65	71.35	71.35
B 4+	37	0.62	<7.0	<7.0	35.7	55.75	83.75
PH 1	203	0.39	<7.0	<7.0	<7.0	20.4	33.2
PH 2	95	0.62	<7.0	<7.0	<7.0	36.8	75.45
PH 3	72	0.67	<7.0	<7.0	28.5	55.75	71.35
PH 4+	34	0.68	<7.0	<7.0	28.6	41.5	62.55