Hypogonadism in females with Prader-Willi syndrome from infancy to adulthood: variable combinations of a primary gonadal defect and hypothalamic dysfunction

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Running title: Ovarian & hypothalamic defects in Prader-Willi Syndrome

Word count: 5990

Key words: Prader-Willi syndrome, Hypogonadism, Ovarian function, Anti-Mullerian hormone, Inhibin B.
ABSTRACT

Objective: The variable hypogonadism in Prader-Willi Syndrome (PWS) has generally been attributed to hypothalamic dysfunction. Recent studies documented primary testicular dysfunction in PWS males. Our aims were to characterize sexual development and reproductive hormones in PWS females and to investigate the etiology of hypogonadism.

Design: a cross-sectional study.

Methods: Physical examination was performed on 45 PWS females (ages 6 weeks to 32 years) and blood samples were obtained for hormonal analyses.

Results: Age of onset and progression of puberty varied; most adults had incomplete sexual development. Spontaneous menarche was reported in 4 (ages 15-30 years) but all had subsequently developed secondary amenorrhea or oligomenorrhea. Anti-Mullerian hormone levels were within the normal range in all age groups. Inhibin B was consistently low or undetectable; only 5 women had levels in the low-normal range (20-54 pg/mL). LH was normal in most children but low (<1.0 IU/L) in 9 of 15 adults. FSH was within the normal range for age in most, but low (<0.5 IU/L) in 10 and high in 4. Estradiol levels were normal-low and androgen levels were normal in the majority.

Conclusions: Pubertal development in PWS females, as in males, is characterized by normal adrenarche, pubertal arrest and hypogonadism due to variable combinations of a unique primary gonadal defect and hypothalamic dysfunction.
INTRODUCTION

Prader-Willi syndrome (PWS), first described in 1956 (1) is a neurodevelopmental disorder with an incidence of around 1/30,000 births in both sexes and in all ethnic groups (2-4). PWS results from the absence of paternal expression of imprinted genes localized in the 15q11-q13. Clinical features, attributed mainly to hypothalamic dysfunction (2, 5-7), include severe hypotonia and feeding difficulties in infancy, an insatiable appetite leading to severe obesity in childhood, short stature, and hypogonadism as well as dysmorphic features and variable degrees of mental retardation (2-4). Although hypogonadism has often been attributed to hypothalamic dysfunction, some studies show that a primary testicular defect contributes to the hypogonadism in PWS males (8-10).

The clinical expression of hypogonadism in females with PWS is variable (2, 3). Genital hypoplasia, delayed and incomplete pubertal development or precocious puberty may occur in both males and females (11). Although some females with PWS undergo spontaneous menarche (7), most have primary or secondary amenorrhea or oligomenorrhea. Nevertheless, pregnancies have been reported in three women with genetically documented PWS (12, 13) indicating that the degree of hypogonadism is unpredictable.

The pathophysiology of hypogonadism in females with PWS has not previously been studied in depth. Recently we found a unique follicular stage-specific defect in 10 women with PWS, suggesting that primary ovarian dysfunction is an important contributor to the hypogonadism in PWS women (14). In order to characterize sexual development in females with PWS and to further investigate the etiology of
hypogonadism, we measured reproductive hormone levels and assessed pubertal
development in a cross-section population of PWS females from infancy to adulthood
followed in our national multidisciplinary PWS clinic.

SUBJECTS AND METHODS

Subjects

In Israel, 94 (48 females) patients are known to have documented PWS, and all are
treated in the national referral, multidisciplinary clinic at Shaare Zedek Medical Center,
Jerusalem. In this report, we describe the findings of 45 of the female patients, ages 6
weeks to 32 years. Three patients declined to participate and their data were excluded
from the study. Partial data on 10 of the adult women presented here were also
described in a separate report (14).

Molecular genetic studies of chromosome 15 confirmed the diagnosis in all patients.
Genetic diagnoses included microdeletion in 27, uniparental disomy (UPD) in 17, and
an imprinting center defect in one. The study was approved by the internal review board
of Shaare Zedek Medical Center. Signed informed consent was obtained from parents,
guardians or adult subjects.

The clinical features of the study population are shown in table 1. Patients were divided
into 4 age groups, roughly corresponding to: A, infancy (birth- 2 years); B, childhood
(3-7 years); C, adolescence (8-16 years); and D, adulthood (17-32 years). At the time of
the study, 3 infants, 9 children and 4 adolescents were receiving growth hormone
treatment. One child and one woman were treated with l-thyroxine for acquired primary
hypothyroidism. Two women had diabetes and were treated with insulin. Other medications included risperdone in 5 women, selective serotnine-reuptake inhibitors (SSRIs) in 5 and topiramate in 2.

**Methods:**

A wall-mounted stadiometer was used for height measurements. Supine length was measured in infants below three years of age. The Growth Analyser version 3.5 software was used to calculate height, weight, and BMI standard deviation scores (sds) using the USA CDC-2000 reference standards. For infants under age two years, weight-for-length sds was used since the CDC-2000 standards do not include BMI data for this age group. Pubertal development was evaluated using the Tanner classification (15, 16).

Serum concentrations of estradiol, LH, FSH, testosterone (T), TSH and prolactin were measured using DxI 800 (Beckman Coulter Instruments Inc., USA). Assay sensitivities were 15pg/ml, 0.1 IU/L, 0.1 IU/L, 0.1ng/ml, 0.03 mIU/L and 0.5ng/ml respectively. The estradiol intra-assay coefficients of variation (CV) were 6.3-15% for levels ≥40pg/ml and 20% for levels <40pg/ml. Inter-assay and intra-assay CVs were less than 7% for other measurements. Dehydroepiandrosterone-sulfate (DHEAS), androstenedione, and sex-hormone binding globulin (SHBG) concentrations were measured using immunochemiluminiscence on IMMULITE (Simens, Diagnostic Product Corporation, Los Angeles, California, USA). Assay sensitivities were 0.1 µg/dl, 0.3 ng/ml and 0.02 nmol/l, respectively and inter- and intra-assay CVs were less than 10%. Serum inhibin B and AMH concentrations were measured using highly sensitive two-site ELISAs (DSL, Webster, Texas, USA). The assay sensitivities were 7 pg/ml and 0.017 ng/ml respectively. Inter- and intra-assays CVs were 15% and 7% for INB and 8.7% and
5.3% for AMH, respectively. Published data from Esoterix Labs (17) were used as normal reference ranges data for hormone levels in children, adolescents and young adults except for AMH, Inhibin B, and SHBG. Age appropriate control data for AMH, Inhibin B, and SHBG were obtained from reference data in the literature (18-22).

**Statistical Analysis**

For statistical analysis, samples with hormone values below the assay detection limit were assigned the detection limit value. Since hormonal levels were not distributed normally, analysis of age related differences was performed using the Kruskal-Wallis test. Comparison between age groups was evaluated with the Mann-Whitney test. The Spearman-rho test was used to examine correlations between hormone levels, SHBG and BMI.

**RESULTS**

**Adrenarche and early puberty**

Breast and pubic hair Tanner stages are shown in Table 2. Although most of the adult women achieved Tanner stage 4 pubic hair and breast development, only five achieved stage 5 pubic hair and three had stage 5 breast development. One 26 year old had no pubic hair (Tanner 1), one 17 year old had only stage 3 pubic hair and three had stage 3 breast developments. Most women had primary amenorrhea; only four had spontaneous menarche (age of appearance 15-30 years); one woman subsequently developed oligomenorrhea and three have secondary amenorrhea. Two women had withdrawal bleeding following progesterone administration at age 15 and 30 years. No correlation
was found between the genetic subtype and the degree of sexual maturation or between weight, BMI or Tanner stage.

None of the girls younger than 9 years at the time of the study showed evidence of true puberty or pubarche. One 9 year-old girl had Tanner 3-4 breast development and Tanner 3 pubic hair. This girl had onset of breast development before her 8th birthday. Her serum FSH, LH and estradiol (10.7 IU/L, 3.3 IU/L and 42 pg/mL respectively) were elevated for her age and her bone age was 11.5 years. A GnRH stimulation test (100 µg iv) showed a peak LH of 13.5 IU/L and a peak FSH of 14.7 IU/l. Serum prolactin was 7.0 ng/mL, testosterone was 2.9 ng/mL, androstenedione was 1.2 ng/ml, DHEAS was 134 µg/dl, and 17- hydroxyprogesterone was 0.8 nmol/l. On abdominal ultrasonography the uterus was 4.5 X 1.1 X 3.5 cm and the ovaries 3.25 X 1.4 X 2.5 and 2.7 X 1.6 X 2.1 cm. Small antral follicles were seen in both ovaries. These findings are consistent with central precocious puberty.

**Hypothalamic-pituitary function:**

Serum levels of LH and FSH are shown in figure 1A-B and table 3. LH levels were normal in childhood and early adolescence (figure 1A, table 3), but was abnormally low (< 1.0 IU/L) in 9 of 15 adults. One 2-year-old who's LH was 15.6 IU/L. All other hormones measured in this girl, including FSH (5.5mIU/mL) were normal. One 21-year-old woman had undetectable levels of both LH and FSH. Four other women with low LH also had abnormally low FSH levels, consistent with severe gonadotropin deficiency.
FSH levels were variable: in 10 patients the values were abnormally low (<0.5 IU/L) and in 8, the values exceeded the upper limit of normal for age (Fig 1B), 2 of them had extremely high levels (a 1 year-old infant whose FSH was 30 and a 15 year-old with FSH of 24.7 IU/L). In both of these patients, FSH levels were consistently high in repeated samples. Both had normal 46,XX karyotypes. FSH changed significantly with increasing age [$\chi^2 (3) = 13.5; P < 0.005$] (Fig. 1B), while no significant changes in LH were found (Fig 1A).

Prolactin levels were within the normal range in most PWS females (fig 1C and table 3); the levels were mildly elevated (25-30 ng/ml) in 2 toddlers and 3 women: a 1 year-old infant with elevated FSH (30mIU/mL), who also had mildly elevated TSH levels (5.6 mIU/L); 2 of the women were treated with risperdone (Fig 1C), a medication known to increase prolactin secretion. Another 3 women treated with risperdone had normal hormonal profiles, including prolactin levels (5.6, 6.2 and 22.5 ng/mL), but TSH levels were mildly elevated in one (6.1 mIU/L). Another 26-year-old woman had a TSH of 6.1 mIU/L.

No correlation was found between the genetic subtype and the hormonal profile.

**Ovarian function**

Serum AMH levels were within the normal range in all participants except for three, whose levels were undetectable (<0.017 ng/mL) (fig 1E and table 3). They included a 15-year-old with high FSH level (24.7 mIU/mL) and undetectable inhibin B, consistent with primary ovarian failure; a 21-year-old with low gonadotropins (FSH 0.4 and LH 0.3 IU/L) and undetectable inhibin B, consistent with hypogonadotropic hypogonadism
and a 31-year-old with normal-low gonadotropins (FSH 0.7 and LH 1.6 mIU/mL) and inhibin B 10 ng/mL, suggesting abnormal regulation of the hypothalamic-pituitary-ovarian axis.

Inhibin B levels were consistently low or undetectable in all age groups (Fig. 1F and table 3). In 24 participants inhibin B was below the lower limit of assay sensitivity (<7 pg/ml); for statistical purposes, these patients were assigned an inhibin B level of 7 pg/ml, although their actual levels were probably even lower. Three women had undetectable serum inhibin B with normal to high FSH (6.7-24.7 IU/L) and 3 had very low inhibin B levels (in the 9 to 10 pg/ml range) along with normal FSH. Four women had undetectable serum inhibin B combined with low (<0.5 IU/L) FSH, consistent with hypogonadotropic hypogonadism.

There was no significant correlation between inhibin B, FSH or AMH levels. No significant correlation was found between inhibin B or AMH and BMI, weight, BMI-sds or weight-sds in all age-groups.

Of the 15 adult PWS women, five had inhibin B levels in the low-normal range (> 20 pg/ml) suggesting partial ovarian follicular development (Table 4 and Fig 1F). Of these five, one had spontaneous menarche at age 19.5, two had primary amenorrhea and 2 had withdrawal bleeding following progesterone challenge. Gonadotropins, estradiol and AMH levels were within the normal range in all five. Inhibin B in these 5 women was the only parameter that was significantly different from the other women (P < 0.001).
Serum estradiol levels were low, albeit within the normal early follicular phase range for all participants (Fig. 1D) and rose significantly with increasing age \[\chi^2 (3) = 19.0; P < 0.001\].

**Androgens**

Serum T, androstenedione and DHEAS levels in most participants were within the normal range (Fig. 1 G-I) and rose significantly with increasing age \([\chi^2 (3) = 22.1, 27.5 and 21.8; P < 0.001]\). T levels were mildly elevated in 10/26 females aged 1-15 years and in 2 of the 15 adults (Fig. 1H). Two women aged 25.5 and 26 years with BMI 52.7 and 49.3 kg/m\(^2\) had hyperandrogenism (T, androstenedione and DHEAS were 0.96 and 0.63 ng/mL, 3.1 and 2.3 ng/mL and 451 and 353 µg/dl and respectively) with high estradiol levels (153 and 50 pg/mL), normal gonadotropins and AMH levels with low inhibin B. Four other children and one 15 year old adolescent had mildly elevated DHEAS, two of whom also had mildly elevated androstenedione levels.

**SHBG**

SHBG levels were below the normal range in 12 of 20 girls (Groups A and B) and in 6 women (Fig. 1J). As in normal subjects, SHBG levels decreased significantly with age \([\chi^2 (3) = 13.8; P < 0.005]\). SHBG correlated significantly with BMI-sds \((r = -0.688; P < 0.001)\).
This study is the first to present a comprehensive description of clinical signs of pubertal development and reproductive hormone levels in a cohort of females with PWS with ages from infancy through adulthood. We found that pubertal development in PWS females, as in males, is characterized by normal adrenarche followed by pubertal arrest. Most females with PWS had variable hypothalamic dysfunction and a unique pattern of ovarian dysfunction characterized by extremely low or undetected inhibin B along with normal AMH levels, resembling the findings of the primary testicular defect in PWS males (10). A small subgroup of women, however, showed detectable inhibin B levels, albeit subnormal for their age, which might be compatible with partial ovarian follicular development.

The dissociation between AMH and inhibin B suggests a stage-specific defect in folliculogenesis in PWS. Although in normal women serum AMH is more strongly related to ovarian follicular status and to fertility potential than serum inhibin B (27), no dissociation between these two TGFβ-superfamily members was found in normal women. During normal folliculogenesis, AMH is secreted from ovarian follicles at primary and secondary preantral immature stages (23), while inhibin B is secreted subsequently from small- and medium-size antral follicles (24-27) in response to FSH stimulation (28). We interpret the findings in PWS to indicate that in most of our patients, the pool of undeveloped ovarian follicles seems to be conserved. The first stages of FSH-independent follicular development occur but subsequent follicular maturation is hampered despite normal to high FSH levels. Estradiol levels were normal-low in most patients, however, serum estradiol is derived from various sources, including peripheral aromatization of adrenal and ovarian androgens and therefore is not a reliable marker of ovarian function.
Hypogonadotrophic hypogonadism was found in more than 25% of the participants in our study, while in males with PWS, severe gonadotropin deficiency is a rare cause of hypogonadism (6, 8-10). The dissimilarity in hypothalamic-pituitary function between males and females with PWS might be explained by the divergence in the regulation of FSH secretion between normal males and females. In both sexes, FSH secretion is stimulated by GnRH and inhibited by inhibin and, in women, also by estrogens. The normal range of inhibin B levels is much higher in males than females at all ages (20, 29-31), estradiol levels in women with normal ovarian function are much higher than in men while FSH levels are similar in both sexes. We suggest that the common cause of hypogonadism in most PWS patients, both males and females, is related to a unique defect in inhibin B secretion. The lack of negative feedback by inhibin B was associated with higher FSH levels in males than in females, in whom estradiol and estrone originates from the abundant adipose tissue, and may contribute to the negative feedback on GnRH and FSH secretion.

The hypothalamic-pituitary-ovarian dysfunction in PWS appears to be unique and differs from other more common disorders. For example, women with PCOS and PWS are obese and amenorrheic; nevertheless their hormone profiles differ. Women with PCOS have high androgens and AMH, normal FSH and estradiol combined with variable inhibin B and LH (36). Furthermore, insulin resistance is a common feature of PCOS (37) but not PWS. Low inhibin B levels are associated with obesity in normal, fertile women (38), but no association between inhibin B and BMI was found in our PWS patients. The hormonal profile of PWS women also differs from that seen in women with either primary (e.g. Kallman's syndrome) or secondary (e.g. anorexia
nervosa) hypogonadotropic hypogonadism in whom, unlike in PWS, AMH is elevated and LH, FSH, estradiol, and inhibin B levels are low (39).

The question of fertility potential in PWS women is yet unanswered. Infertility due to hypogonadism has been thought to be a consistent feature in PWS (2-4). However, three case-reports of pregnancies in genetically-proven PWS have been published (12, 13), including one with Angelman syndrome in the offspring (13). Seven more pregnancies in two women in whom PWS was diagnosed only by clinical criteria were reported before the genetics of PWS had been delineated (32). Another woman with chromosome 15q11-q13 deletion gave birth to two children (33). In fact, pregnancy in women with PWS may be less rare than commonly thought. Since inhibin B levels, in normal women, are known to correlate with ovulation and potential fertility (27, 28), we suggest that the five PWS women in our study who had inhibin B levels approaching clinical significance [20.3-47.3 pg/ml, normal range 20-260 pg/ml (21)], may comprise a subset of PWS women who are potentially fertile.

The variability of hormonal patterns in the current study is consistent with the reported diversity of ovarian morphology (13, 14, 34). Although ultrasonography in 10 PWS women showed significantly lower uterine and ovarian volume and antral follicle count (AFC) compared to controls (14), there was substantial morphological heterogeneity within the group: AFC ranged from 0-18 follicles, uterine volume from 5-32 ml and ovarian volume from 0.9-5.3 ml. There are only two reports on ovarian histology in PWS. One, in a 21-y-old woman at autopsy, showed no follicle development (33). The second was obtained during cesarean section, showing normal follicles in all stages of development (13).
Normal appearance and development of pubic hair ("adrenarche" or "pubarche") suggests unimpaired adrenal androgen secretion, corresponding to the observed normal androstenedione and DHEAS levels. Sexual development was incomplete, however, in most PWS patients, consistent with arrested pubertal development. By contrast, otherwise healthy obese girls, usually undergo normal or early pubertal development (35).

The genetic mechanisms responsible for hyogonadism in PWS have not yet been elucidated. Studies on the mouse chromosome 7C, which is the homolog of human 15q11-q13, have shown that expression of its imprinting center transcript is abundant in the brain and in the ovary, particularly in oocytes and granulosa cells of the secondary and developing follicles, while no expression was found in other tissues (40). Other studies in the mouse suggest that loss of the necdin gene in chromosome 7C may result in impaired development of GnRH neurons (41). The expression in ovary may be important for other factors necessary for oocyte/follicle development or inhibin B secretion.

In conclusion, PWS females have a unique hormonal profile indicating a follicular stage-specific primary ovarian defect resulting in early arrested maturation of the ovarian follicles. This defect is similar to that of the testicular dysfunction in PWS males, although the relative contributions of hypothalamic and primary gonadal dysfunction vary among males and females. Most women with PWS are likely to be infertile due to combined primary hypogonadism and variable hypothalamic dysfunction. However, follicular development, as indicated by normal (albeit low-normal) inhibin B secretion in a minority of patients may indicate that they are
potentially fertile. Evaluation of hypogonadism by physical examination, hormonal profile and particularly measurement of serum inhibin B in adolescents and women with PWS may be helpful as part of overall clinical management. Whether to recommend treatment with hormone replacement therapy (estrogen and/or cyclic progesterone) or contraception should be considered on an individual basis.

**Declaration of interest:**

This work was supported by grants from Pfizer Pharmaceuticals and from the Prader Willi Syndrome Association (PWSA USA).

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
REFERENCES


FIGURE LEGENDS

Figure 1A-1J:
Serum concentrations of LH, FSH, prolactin (PRL), estradiol, anti-Mullerian hormone (AMH), inhibin B, androstenedione, testosterone, sex-hormone binding globulin (SHBG), and dehydroepiandrosterone-sulfate (DHEAS) in PWS females, plotted as a function of age. Upper and lower limits of normal are indicated by solid lines. Medians for inhibin B and AMH are indicated by dotted lines; the lower limits of normal are lower than the assay sensitivities for some age groups. Prolactin levels (figure 1C) in patients treated with risperidone appear as open circles.
Table 1: Clinical features of 45 females with PWS

<table>
<thead>
<tr>
<th></th>
<th>Group A (Infancy)</th>
<th>Group B (Childhood)</th>
<th>Group C (Adolescence)</th>
<th>Group D (Adulthood)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>7</td>
<td>13</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Age (yr) range (mean ± SD)</td>
<td>0.1-2 1.1 ± 0.6</td>
<td>3-7 5.1 ± 1.3</td>
<td>8-16 10.5 ± 2.2</td>
<td>17-32 23.5 ± 4.5</td>
</tr>
<tr>
<td>Height-sds (mean ± SD)</td>
<td>-0.97 ± 0.76</td>
<td>-1.77 ± 1.61</td>
<td>-0.49 ± 0.64</td>
<td>-2.89 ± 1.60 (^b)</td>
</tr>
<tr>
<td>BMI-sds (mean ± SD)</td>
<td>-1.18 ± 1.62</td>
<td>1.35 ± 1.21 (^a)</td>
<td>2.02 ± 0.72</td>
<td>1.71 ± 1.05 (^*)</td>
</tr>
<tr>
<td>Genetic subtype (DEL/UPD/IMP)</td>
<td>6/1/0</td>
<td>8/5/0</td>
<td>5/5/0</td>
<td>8/6/1</td>
</tr>
</tbody>
</table>

Values are mean ± SD for age in years, height-sds, and BMI-sds for each age group.

* Actual BMI in Group D = 37.2 ± 11.9 (range 20.3-55.0) kg/m\(^2\).

Genetic diagnoses are indicated by DEL = deletion; UPD = uniparental disomy; IMP = imprinting center defect.

\(^a\) Group B differed significantly from group A, P < 0.005.

\(^b\) Group D differed significantly from group C, P < 0.0005.
### Table 2: Pubertal development in PWS females

<table>
<thead>
<tr>
<th></th>
<th>Group A (≤2 years)</th>
<th>Group B (3-7 years)</th>
<th>Group C (8-16 years)</th>
<th>Group D (&gt;16 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pubic hair Tanner stage</td>
<td>1 (1 – 1)</td>
<td>1 (1 – 3)</td>
<td>2 (1 – 3)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4 (1 – 5)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Breast Tanner Stage</td>
<td>1 (1 – 1)</td>
<td>1 (1 – 2)</td>
<td>2 (1 – 3)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4 (3 – 5)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Menarche Numbers (percent)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4&lt;sup&gt;c&lt;/sup&gt; (31)</td>
</tr>
</tbody>
</table>

Values are medians (range).

<sup>a</sup> Group C differed significantly from group B for Tanner pubic hair (P < 0.01) and breast (P < 0.001) stages.

<sup>b</sup> Group D differed significantly from group C for Tanner pubic hair (P < 0.005) and breast (P < 0.0001) stages.

<sup>c</sup> Four women had spontaneous menarche; 2 others had withdrawal bleeding following progesterone treatment. Missing data for 2 women.

Median age of transition to Tanner stage 2 breast developments and pubic hair in a USA population of normal white girls range from 10.0 to 10.4 and 10.5 to 10.6 years respectively; median age of transition to Tanner 5 breast development and pubic hair are 15.5 years and 16.3 respectively (42). Median age of menarche in USA white girls according to studies reported between the years 1997 and 2003 ranges from 12.6 to 12.9 years (42).
Table 3: Hormone levels in PWS females

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Group A (&lt;2 years)</th>
<th>Group B (3-7 years)</th>
<th>Group C (8-16 years)</th>
<th>Group D (&gt; 16 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (mIU/mL)</td>
<td>3.5±5.8</td>
<td>0.38±0.32</td>
<td>1.1±1.9</td>
<td>2.4±2.5</td>
</tr>
<tr>
<td>FSH (mIU/mL)</td>
<td>9.1±9.5</td>
<td>1.0±0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.6±7.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.0±3.8</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>&lt;15</td>
<td>&lt;15</td>
<td>18.6±19.3</td>
<td>39.0±36.9</td>
</tr>
<tr>
<td>Prolactin (ng/mL)</td>
<td>14.6±6.9</td>
<td>10.4±6.3</td>
<td>10.2±4.9</td>
<td>13.4±9.0</td>
</tr>
<tr>
<td>AMH (ng/mL)</td>
<td>0.77±0.67</td>
<td>1.53±1.94</td>
<td>1.61±1.51</td>
<td>1.22±0.77</td>
</tr>
<tr>
<td>Inhibin B (pg/mL)</td>
<td>13.3±13.6</td>
<td>7.3±0.9</td>
<td>8.2±1.6</td>
<td>17.0±15.4</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>129.0±66.2</td>
<td>73.5±50.1</td>
<td>39.6±20.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.3±20.4</td>
</tr>
<tr>
<td>Testosterone (ng/mL)</td>
<td>0.12±0.05</td>
<td>0.16±0.09</td>
<td>0.30±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.41±0.20</td>
</tr>
<tr>
<td>Androstenedione (ng/mL)</td>
<td>0.42±0.27</td>
<td>0.35±0.12</td>
<td>0.86±0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.41±0.70&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DHEA-S (mcg/mL)</td>
<td>16.8±6.2</td>
<td>52.2±48.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>130.2±90.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>196.9±111.6</td>
</tr>
</tbody>
</table>

Data represent mean ± SD of hormone levels in females with PWS.

<15 - levels in all patients were below assay sensitivity.

<sup>a</sup> Group B values differed significantly from group A values for FSH, P < 0.001 and DHEAS, P < 0.04.

<sup>b</sup> Group C values differed significantly from group B values for FSH, P < 0.02; testosterone, P < 0.002; SHBG, P < 0.05; androstenedione, P < 0.001 and DHEAS, P < 0.02.

<sup>c</sup> Group D values differed significantly from group C values for estradiol, P < 0.04 and androstenedione, P < 0.04.
Normal ranges for LH: prepubertal girls ages 1–8 yr, 0.02–0.3 mIU/ml; girls ages 10–14 yr, 0.1–12.0 mIU/ml; and adult females, 2.0–9.0 mIU/ml (18).

Normal ranges for FSH: prepubertal girls ages 1–8 yr, 1.0–4.2 mIU/ml; girls ages 10–14 yr, 1.5–12.8 mIU/ml; and adult females, 1.8–11.2 mIU/ml (18).

Normal reference ranges for prolactin for girls and adult females, 3–24 ng/ml (18).

Normal ranges for estradiol: prepubertal girls ages 1–10 years, less than 15 pg/ml; girls ages 10–14 years, 7–60 pg/ml; and adult females, 30–75 pg/ml (18, 21, 22).

Normal values (range, mean) for AMH in girls: ages 0-1 year, 0.2 – 1.9, 0.66 ng/ml; 1 – 2 years, 0.2–3.9, 0.9 ng/ml; ages 4 – 6 years, 0.1-8.1, 1.4 ng/ml; ages 8 – 10 years, 0.2 – 8.9, 2.3 ng/ml; ages 12 – 14 years, 0.3-8.6, 3.1 ng/ml; ages 16-18 years, 0.2 – 8.1, 2.1 ng/ml; and ages 18 – 32 years, 0.2 – 7.0, 1.7 ng/ml (19, 20).

Normal ranges for inhibin B in girls (range, median, % undetected): 0- 6 years, <7 – 73, 11 pg/ml, 35%; 6 – 10 years, <7 – 130, 21 pg/ml, 6%; 10 –11 years, 20 – 186, 68 pg/ml, 0%; 12 – 18 years, 14 – 362, 99 pg/ml, 0% (21, 22); adult women aged 21 – 32 in early follicular phase, 20 - 261 pg/ml (22).

Normal ranges for testosterone: prepubertal girls ages 1–10 yr, 0.03–0.10 ng/ml; girls ages 10–14 yr, 0.15 –0.35 ng/ml; and adult females, 0.1–0.55 ng/ml (18).

Normal ranges for SHBG (18): 72–220 nmol/liter for ages 2–8 yr; 36–125 nmol/liter for pubertal age girls; and 40–120 nmol/liter for adult females. Mean (range) values for normal girls (23), 113 (53–240) nmol/liter for girls ages 6–7 yr; 100 (42–239) nmol/liter for ages 8–9 yr; 81 (33–196) nmol/liter for ages 11–12 yr; 68 (28-166) nmol/liter for ages 13–14 yr; 59 (21–168) nmol/liter for ages 16–17 yr; and 57 (14–236) nmol/liter for ages 19–20 yr.

Normal mean values (ranges) for androstenedione: normal girls, ages 1–10 yr, 0.24 (0.08–0.50) ng/ml; girls ages 10–14 yr, 1.23 (0.8–1.9) ng/ml; adult females, range, 0.60–2.45 ng/ml (18).
Reference ranges for DHEAS: for children ages 1–5 yr (<5–57) mcg/dl; 8–10 yr, 0.13–1.15 mcg/dl; 10–15 yr, 32–226 mcg/dl; and for adults the normal range is 76 – 226 mcg/dl (18).
Table 4: Five PWS women with low-normal range inhibin B levels

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Age</th>
<th>Genetics</th>
<th>Menarche (age)</th>
<th>Pubic hair Tanner</th>
<th>Breast Tanner</th>
<th>Weight (kg)</th>
<th>BMI (kg/m^2)</th>
<th>E2 (pg/ml)</th>
<th>FSH (IU/L)</th>
<th>LH (IU/L)</th>
<th>AMH (ng/ml)</th>
<th>INB (pg/ml)</th>
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<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>DEL</td>
<td>No</td>
<td>5</td>
<td>3</td>
<td>56</td>
<td>23.9</td>
<td>13.4</td>
<td>3.1</td>
<td>0.5</td>
<td>2.0</td>
<td>31.2</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>DEL</td>
<td>19.5</td>
<td>5</td>
<td>4</td>
<td>78.9</td>
<td>35.1</td>
<td>34.6</td>
<td>6.8</td>
<td>4.1</td>
<td>0.762</td>
<td>47.3</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>UPD</td>
<td>No</td>
<td>4</td>
<td>5</td>
<td>123.8</td>
<td>55.0</td>
<td>22.6</td>
<td>8.9</td>
<td>5.4</td>
<td>0.99</td>
<td>20.6</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>DEL</td>
<td>following P</td>
<td>4</td>
<td>4</td>
<td>49</td>
<td>23.3</td>
<td>60.8</td>
<td>6.3</td>
<td>5.9</td>
<td>0.75</td>
<td>54</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
<td>DEL</td>
<td>30y, following P</td>
<td>5</td>
<td>5</td>
<td>110</td>
<td>53.1</td>
<td>50.7</td>
<td>10.0</td>
<td>5.6</td>
<td>1.12</td>
<td>20.3</td>
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