

## DETAILED NARRATIVE

**Title:** Gonadotropin Releasing Hormone (GnRH) Agonist Test in Disorders of Puberty

**IRB Number:** 13472A

**Principal Investigator:** Robert L. Rosenfield, M.D., Professor of Pediatrics and Medicine, Pediatric Endocrinology

### **A. Background and Prior Pertinent Experimental Findings:**

The following is an explanatory overview of the enclosed detailed, referenced research proposal in USPHS grant proposal format.

#### **1. GnRH and GnRHag Testing in Relationship to Pubertal Development.**

Gonadotropin releasing hormone (GnRH) is the hormone made in the hypothalamus that stimulates specific cells in the pituitary gland (gonadotropes) to make and release gonadotropins. It is made in pulses at about 1.5 hour intervals. Gonadotropins are the hormones that regulate the testicles and ovaries. There are two gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH is a short-acting hormone that is responsible, in conjunction with FSH, for stimulating production of gonadal hormones, such as the sex hormones estradiol and testosterone. FSH is a more long-acting hormone that stimulates egg and sperm development, in conjunction with LH.

Increasing production of GnRH brings about puberty by stimulating increasing pituitary secretion of LH and FSH. The gonadotropin response to GnRH, particularly that of LH, increases as puberty progresses because the body's internal production of GnRH enhances the gonadotropin response to subsequent GnRH stimulation. Mature production of GnRH, LH, and FSH is required for the mature function of the regular menstrual cycle in females, with cyclic ovulation, and for the production of sperm in males. The pituitary only responds to an optimal amount of GnRH delivered in an optimal pulsatile manner: too little GnRH or GnRH pulses too far apart do not stimulate mature pituitary gonadotropin output; too much GnRH or GnRH delivered too frequently paradoxically inhibits pituitary gonadotropin output by desensitizing it, i.e., preventing it from "recharging" after discharging the pituitary stores of gonadotropin.

The hypothalamic-pituitary-gonadal axis is in place before birth and capable of functioning at any age. However, the central nervous system of young children suppresses its function. This neural restraint normally begins to wane at about the end of the first decade of life, which allows puberty to commence.

The first hormonal change of puberty is sleep-associated GnRH secretion, which is most sensitively indexed by blood levels of the gonadotropin LH. During early puberty, this sleep-associated LH increase is transient. As puberty advances, it lasts longer and longer into the day until eventually there is no diurnal rhythm when puberty is complete. As a consequence of increasing GnRH secretion as puberty progresses, the pituitary gonadotropes become more sensitive to successive exposure to GnRH, whether it is of endogenous or exogenous origin.

The responses of LH and FSH to a GnRH challenge test increase significantly with advancing pubertal stage, in both normal and abnormal puberty. In addition, because GnRHag provides a more prolonged stimulation of the gonadotrope, the responses of gonadal hormones increase with pubertal stage in parallel with those of LH and FSH.

GnRH itself (Factrel®) is the standard diagnostic agent used by pediatric endocrinologists to

attempt to diagnose disorders of puberty since assessment of sleep-associated LH secretion is not practical. A standard Factrel test dose stimulates the release of preformed LH and FSH (the readily releasable pool). This is best assessed by assaying the transient peak of pituitary LH which occurs at 30-60 min (2). In contrast, a single dose of a synthetic GnRH agonist (GnRHag) additionally creates a “reserve pool” of LH and FSH by stimulating their synthesis; LH and FSH release then peaks at 4 hours and persists for 24 hours. This is sufficient to stimulate sex hormone secretion within 24 hours in both men and women.

These observations suggested that a GnRHag diagnostic test for pituitary-gonadal function may make it superior to GnRH itself as a test agent for diagnosing disorders of puberty. Initial studies with the injectable GnRHag nafarelin supported this concept. However, nafarelin injection was never brought to market because its manufacturer (Syntex) was bought out by a company (Searle) that had no interest in diagnostic agents. Leuprolide acetate, a short-acting version of the long-acting leuprolide depot preparation (Lupron Depo/Ped®) that has been approved for the treatment of sexual precocity in children, has been shown to yield a degree of pituitary-gonadal stimulation comparable to nafarelin.

The Food and Drug Administration (FDA) funded pilot studies of nafarelin and leuprolide under IND's #40,387 and 60,003, respectively, for diagnostic testing for gonadotropin deficiency (FD-R-001012 and FD-R-001473). However, the manufacturer of Lupron® has not been interested in the diagnostic potential of leuprolide injection since the diagnostic market is relatively small.

There are two additional obstacles to making a timely diagnosis of disorders of puberty. One is manufacturing cut-backs for low-profit drugs. This has led to frequent unavailability of Factrel for testing purposes, so pediatric endocrinologists have been forced to turn to leuprolide acetate for diagnostic testing even though norms have not been well-established. Second is that advances in gonadotropin assay methodology have led to diagnostic criteria for disorders of puberty being in a state of flux. Gonadotropins are complex polypeptides that are made up of a combination of a relatively nonspecific alpha subunit with a specific LH beta- or FSH-beta subunit. Furthermore, gonadotropins are glycoprotein hormones that exhibit molecular heterogeneity because of varying degrees of glycosylation; the gonadotropin standards themselves differ because of this. Within the past decade “third generation” immunometric assays of high sensitivity and high specificity that are monoclonal antibody-based have become available for diagnostic purposes to widely replace the more variable polyclonal radioimmunoassay previously available.

Standardizing the diagnostic use of a GnRHag challenge test for disorders of puberty, thus, calls requires a research approach. The most common disorders of puberty are premature (precocious) puberty and delayed puberty. Both central precocious puberty and gonadotropin deficiency, the cause of delayed puberty that is most difficult to diagnose before adulthood, are rare in the general population, and so they are designated as “orphan disorders” by the FDA. However, sexual precocity and delayed puberty are commonly encountered in pediatric practice. A survey in pediatricians' offices showed that at age 7 years, 27% of African-American girls and 6.7% of White girls had breast or pubic hair development and at age 8 years these figures had respectively risen to 48% and 14%. Approximately 5% of boys do not show signs of puberty by 14 years of age. We propose to evaluate GnRHag as a means to assist in differentiating among the most common causes of precocious puberty and of delayed puberty in children and adolescents. In both situations, a sleep test (defining LH characteristics during sleep) will be the immediate independent variable; in the case of delayed puberty, the final distinction between

constitutional delay in puberty (CDP, a variation of normal) and gonadotropin deficiency (GnD, a medical disorder) will be on the basis of the progress of puberty over time. Our preliminary studies show that other pituitary gonadotrope products (free alpha subunit, FAS) (unpublished) and gonadal hormones (inhibin-B) are also released into the circulation with a leuprolide challenge. FAS appears to be a more accurate end-point than LH for the GnRHag test in distinguishing delayed puberty from gonadotropin deficiency. Collaborative preliminary studies (**Preliminary Studies**) now show that other pituitary gonadotrope products (free alpha subunit, FAS) (unpublished) and gonadal hormones (activin-A, inhibin-B) (10) that are also released into the circulation are promising outcome variables.

## **2. Premature (Precocious) Puberty**

Complete (true) precocious puberty is gonadotropin-dependent). It results from the premature activation of the hypothalamic-pituitary-gonadal axis. Pituitary LH and FSH are produced at pubertal levels at an excessively young age. This causes inappropriate levels of sex steroid secretion: the complications include permanent short stature from premature closure of the epiphyseal growth plates of bones, psychosocial consequences, and, in girls, premature menstruation. It can occur as a consequence of organic disorders of the brain, as a consequence of other disorders that advance somatic maturity, or be idiopathic. It can occur on an autosomal dominant basis, with apparent incomplete penetrance, particularly in boys; the underlying genetic defects have not been identified. The treatment includes desensitization of the pituitary to GnRH by the use of a long-acting GnRHag, such as Lupron Depo/Ped®, to inhibit endogenous GnRH secretion and, hence, puberty.

This must be distinguished from incomplete (pseudo-) precocity. This is gonadotropin-independent premature sexual development, and output of LH and FSH is low. It may arise from minimal activation of the ovaries, as in the common normal variant, idiopathic premature thelarche. More unusual but serious disorders of the gonads or adrenal glands are the major item in the differential diagnosis. These include feminizing or virilizing tumor, virilizing congenital adrenal hyperplasia, McCune-Albright syndrome, and primary Leydig cell hyperplasia. These disorders cause the same complications as complete precocity and, indeed, may cause complete precocity. These disorders require different kinds of treatment.

## **3. Delayed Puberty**

CDP is a persistence of the physiologically low gonadotropin levels of childhood into the teenage years. It can be extremely difficult to distinguish teenagers with CDP from those with an abnormal degree of gonadotropin deficiency (GnD). However, CDP children are “late bloomers” who spontaneously enter puberty in their late teens and go on to grow and mature normally. Gonadotropin deficiency can be congenital, hereditary, or acquired. Genetic causes of isolated GnD account for less than 10% of cases. Acquired GnD can be organic, as in the case of brain or pituitary tumor, or be functional, as in anorexia nervosa or hypothalamic amenorrhea (a transient form of the latter is “boarding school amenorrhea”). GnD requires long-term sex hormone replacement therapy and, eventually, fertility treatment.

## **B. Purpose of the Study:**

### **1. Hypotheses**

The hormonal responses to injection of a challenge dose of GnRH agonist (GnRHag) will distinguish among disorders of puberty as well as a sleep test. Specifically, we will test the hypotheses that the response to injection of the GnRH agonist leuprolide acetate will:

- a. Distinguish among the causes of precocious puberty

- b. Distinguish among the causes of delayed puberty.

## 2. Potential Knowledge to be Gained

The main goal is to establish the diagnostic effectiveness of a GnRHag test and norms for it. This will improve the differential diagnosis of the most common disorders of puberty so that we may provide more accurate and earlier treatment for these disorders.

## **C. Description of Protocol Methodology:**

### 1. Conduct of Study

a. **Admission procedures.** The subject will be admitted to the General Clinical Research Center (GCRC) at the University of Chicago Hospitals at approximately noon on day 1. Admission procedures will be performed: history and physical examination will be performed by the admitting Pediatric Endocrinology (General Pediatric) Service; this will include height, weight, and pubertal staging.

b. **Sleep test.** An intravenous line will be inserted at 6 PM. Blood sampling will commence at approximately 7 PM and continue until 7 AM. Blood will be collected in aliquots for hormone assays at sequential 20-min intervals from an indwelling nonthrombogenic intravenous line by constant withdrawal pump.

c. **GnRH agonist test.**

- 1) Blood sampling will commence at approximately 0700 hr with baseline blood samples obtained at 20 min intervals x 4 (-60 to 0 time). The same line will be used if possible.

- 2) Leuprolide acetate injection: 10 mcg/kg subcutaneous at 0 time.

- 3) Blood sampling will continue after the leuprolide dose: 0.5, 1, 2, 3, 4, 8, 12, 16, 20, and 24 hr. LH and FSH will be measured in all samples; testosterone (boys) and estradiol (boys and girls) at 0, 16, 20, and 24 hr. Extra blood will be obtained at 4 and 24 hr for special studies (e.g., FAS, inhibin-B)

d. **Miscellaneous procedures.**

1. Bone age radiograph will be obtained if not performed within 3 months.

2. Blood will be withdrawn (15-30 cc) for DNA.

3. Discharge on prophylactic ferrous sulfate (300 mg daily for 1 month).

### 2. Data Collection

Hormone assays will be performed in the University of Chicago Hospital Endocrine Laboratory using commercially available assays.

Serum will be stored for assay of inhibin-B, activin, and FAS.

Molecular genetic studies. Blood (5-10 cc x 3) will be collected to extract DNA, prepare a lymphoblastoid cell line, and freeze in the GCRC Core Laboratory for potential studies of the molecular genetic basis of disorders of puberty. One of these tubes will be used for the creation of an EBV-transformed lymphoblastoid cell line which may be useful as a permanent source of DNA.

## **D. Probable Duration of Protocol:**

Five years.

## **E. Exact Location Where Research is to be Conducted:**

The study will be conducted in the General Clinical Research Center of the University of Chicago Hospitals, which is located on corridor W5 in the Bernard Mitchell Hospital.

#### **F. Special Precautions to be Taken by Researchers:**

Children are hospitalized directly across from the nursing station in private pediatric rooms with locked windows and have a bed-chair for a parent to stay, which is encouraged. Only a parent or guardian may take a child off the research unit. A fully stocked resuscitation cart is kept on the GCRC unit.

See also “**Procedures for Minimizing Potential Risk.**”

#### **G. Description of Experimental Controls and Use of Placebos:**

1. Control subjects will be healthy volunteers. Study groups will be constituted as follows:
  - a. Prepubertal children: 20 boys (9-13 years old) and 20 girls (8-12 years old)
  - b. Early pubertal children: 20 boys and 20 girls with chronologic and bone ages of 9-15 years of age.
2. Placebos will not be used in this study.

#### **H. Type and Number of Experimental Subjects, Including Method of Subject Selection, Randomization and Inclusion and Exclusion Criteria:**

1. Experimental subjects: type, number, and inclusion criteria.

##### **Re Specific Aim 1: Premature Puberty**

Premature puberty is defined as breast development of onset from 6 months to 8 years of age (girls) or pubic hair development or testicular enlargement of onset from 6 months to 9 years of age (boys) in associated with bone age advancement of > 2 S.D. Blood volume considerations preclude study of children under 10 kg.

- 1) Premature thelarche, idiopathic: 20 girls, under 8 years of age, with weight >10 kg.
  - Inclusion criteria: breast development as an isolated phenomenon before 8 years of age, bone age within 2 S.D. of average for age, and plasma estradiol levels below 9 pg/ml.
- 2) Complete (gonadotropin-dependent) sexual precocity: 20 children of each sex, under 8 (girls) or 9 (boys) years of age, with weight >10 kg.
  - Inclusion criteria: pubertal sex steroid levels (estradiol over 9 pg/ml in girls and testosterone over 20 ng/dl in boys) with bone age 2 SD or more advanced. The diagnosis will be confirmed by this study to include pubertal sleep-related LH increases.
- 3) Gonadotropin-independent precocity -- 20 children of either sex, under 8 (girls) or 9 (boys) years of age, with weight >10 kg. They will have such disorders as McCune-Albright syndrome, primary Leydig cell hyperplasia, tumors, and congenital adrenal hyperplasia.
  - Inclusion criteria: The diagnosis of these disorders will be established independently by the pattern of sex hormone secretion, absence of sleep-related LH rises, and ultrasound or other radiologic imaging procedures as indicated clinically.

##### **Re Specific Aim 2: Delayed Puberty**

Delayed puberty criteria will be retardation of both pubertal milestones and bone age by two or more years at 14 through 17 years of age.

- 1) Constitutional delay of puberty (CDP): There will be 2 groups of prepubertal children, consisting of 20 boys and 10 girls, and 2 groups of early pubertal children (20 boys and 10 girls).
  - Inclusion criteria: These children will be otherwise healthy (see Exclusion criteria). The diagnosis will be supported independently initially by sleep test criteria in this protocol and confirmed by spontaneous progress of puberty upon follow-up.
- 2) Gonadotropin deficiency (GnD): There will be 2 groups of prepubertal patients, consisting of 20 males and 20 females, and 2 groups of pubertal children with partial GnD who are arrested in pubertal development, consisting of 20 males and 20 females.
  - Inclusion criteria: Since GnD is a rare (“orphan”) disorder, adult patients will be included in this study population for comparison. The diagnosis of GnD will be provisionally assigned to teenagers if delayed puberty is associated with (1) anterior panhypopituitarism, (2) a hypothalamic-pituitary mass upon magnetic resonance imaging (MRI), (3) cranial irradiation therapy, (4) anosmia ± MRI evidence of Kallmann's syndrome, or (5) congenital micropenis without evidence of primary hypogonadism. Confirmation of the diagnosis of GnD will be by lack of onset of puberty by 18 years of age in males (17 years in females) or lack of spontaneous progression of puberty, upon re-evaluation within 1 year after completion of one or more courses of replacement sex steroid therapy.

## 2. Exclusion criteria

- a. Constitutional delay of puberty: Chronic systemic, metabolic, and endocrine disease will be excluded by history, physical examination, complete blood count, erythrocyte sedimentation rate, comprehensive metabolic panel, thyroxine, and somatomedin-C determinations.
- b. All groups: Sex hormone usage within 2 months.

## 3. Method of Subject Selection

- a. Patients will be those presenting to the Pediatric Endocrinology Clinics of the University of Chicago Medical Center. The disorders under study are uncommon, and the literature cited in **Background** does not suggest any unusual demographic distribution. We will attempt to recruit all subjects meeting eligibility criteria by speaking with them directly in the clinic. African-American girls appear to be at particular risk for premature puberty. Otherwise, the demographics of the study population will reflect the demographics of the US population as determined in the 1990 US Census, i.e., 0.8% Alaskan/American Indian, 2.9% Asian, 12.1% African-American, 9.0% Hispanic, 80.3% Caucasian, (some minorities double-counted). Our accrual to the immediate predecessor versions of this protocol has been 1% Asian, 24% Afro-American, 7% Hispanic, and 67% White, with 60% boys and 40% girls (n=88).
- b. Control children are recruited by IRB-approved advertisement with the goal of frequency-matching the age, stage, and ethnicity of the patients.

## 4. Randomization.

There is no randomization in this study.

## **I. Description of Statistical Analysis to which Data will be Subjected:**

### **1. Variables**

a) Sleep test. The gold standard test for the diagnosis of GnD will be demonstration of a subnormal sleep-associated increase in LH level. The onset of true puberty will be defined as a significant sleep-related rise in LH ( $\Delta$ LH, mean sleep minus mean pre-sleep) to, provisionally, 0.35 IU/L or more; normal  $\Delta$ LH for pubertal children will be, provisionally, 0.8 IU/L IU/L or more. Normal LH pulse frequency is defined as  $> 2$  pulses/6 hr.

b) GnRHag test. Plasma concentrations of LH, FSH, estradiol and/or testosterone in samples collected before and after GnRHag. Baseline, "early" (30-60 min), 4 hr, and peak values will be analyzed as levels and responses ( $\Delta$ ). The primary variable for the delayed puberty study will be the FAS response at 4 hr.

### **2. Comparison of groups**

a) Either the 5th or 95th percentiles of the separate prepubertal and pubertal groups of healthy volunteers will define the lower or upper limit of normal range for the test, depending upon the study group in question. This fixes the specificity of the test at 95%. A similar range will be constructed for girls with central precocious puberty and CDP boys.

b) Re premature puberty. Variables will be compared among the controls and the subgroups of patients with premature puberty to determine the statistical significance of differences (by analysis of variance and unpaired t-tests, as appropriate, with correction for multiple comparisons where indicated). We expect that variables for patients with complete precocious puberty will not be significantly different than those of sex-matched pubertal controls. If so, the data for patients with idiopathic precocious puberty will be pooled with that of the healthy controls of appropriate sex.

c) Re delayed puberty. The responses of normal prepubertal boys will be compared to those of prepubertal CDP and GnD boys, and likewise early pubertal normal, CDP, and GnD boys will be compared. The mean, S.D., and shape of the distribution curve will be determined for each variable above for each test (sleep, GnRHag). Responses will be compared to determine whether differences among the prepubertal groups (normal, CDP, GnD) are statistically significant ( $p < .05$ ) by analysis of variance, with post-hoc Sheffe's test to correct for multiple comparisons, and likewise for the comparisons among the early pubertal groups. Girls' responses will be similarly analyzed. However, because so few girls present with CDP, if the normal and CDP prepubertal groups are not significantly different, their data will be pooled into a prepubertal control group, and likewise with the respective early pubertal groups. Then two-sample t-tests will be used for comparison of prepubertal and early pubertal GnD girls with pooled prepubertal and early pubertal control groups, respectively.

### **3. Specificity and sensitivity of GnRHag test for diagnosis of complete precocity**

We expect to see little overlap between the variables for complete precocious puberty and healthy prepubertal controls for either sex.

### **4. Specificity and sensitivity of GnRHag test for diagnosis of GnD**

a) For boys the principal control group will be the stage-matched CDP patients. CDP will be provisionally diagnosed based on the sleep test. The 5th percentile will be calculated for each variable at each time point separately in prepubertal and early pubertal CDP boys. The sensitivity of the GnRHag test will be determined by the fraction of GnD patients lying below the 5th percentile of the stage-matched control group. For girls the principal control group will

be the normal volunteers, or the pool of normals and CDP, since so few girls present with CDP.

b) Upon conclusion of the study, after the final categorization of patients as GnD or CDP by the criterion of progression of puberty, the specificity and sensitivity of the GnRHag test will be compared with those of the sleep test. We expect that the sleep test and GnRHag test will have equivalent sensitivity in the diagnosis.

## **J. Potential Risks and Benefits to Subjects**

### **1. Potential Risks**

- a. The needle is ordinarily left in place for 36 hours and may cause irritation or bruising at the insertion site, redness or swelling of the vein, or infection. Precautions will be taken to prevent this. The intravenous line may be removed during part of this time if that is the child's preference or if there are access limitations; removal of the line is more common in younger children.
- b. The amount of blood taken is equal to less than a cup (8 ounces) total. This amount of blood is ordinarily not enough to cause a low blood count. Although the amount of blood drawn is not dangerous, it may be enough to cause temporary dizziness. Precautions will be taken to prevent this.
- c. Heparin may be used to prevent clotting in the IV tubing. The amount of heparin used is too little to cause excessive bleeding from the vein.
- d. Anxiety symptoms may occur related to the needle sticks required to draw blood samples. These include numbness or tingling of the hands or feet, constipation.
- e. Leuprolide has no known direct or permanent side effects. In adult women the changes in hormones may cause temporary premenstrual-type symptoms and/or delay ovulation by about a week after the test is performed. Premenstrual symptoms include nausea, breast tenderness, and mood swings. Delayed ovulation can delay the onset of the next period. Leuprolide is mixed in a preservative called benzyl alcohol to which some people are allergic. Allergic reactions vary from one person to another and may include redness, rashes, and swelling. have performed 457 leuprolide diagnostic tests in children and adults with no adverse events.

The other side effects reported from leuprolide use are from chronic use in adults, generally from the depot preparation, and are the expected side effects of the major effect of the drug, which is to turn off ovarian function. This leads to menopausal symptoms, such as hot flashes, and decreased sexual ability. As these side effects are not relevant to the preparation and dosage in use in this study, they are not mentioned in the consent form.

- f. Unforeseeable side effects or adverse reactions, such as allergic reactions could occur. A severe allergic reaction has been reported after man-made GnRH was given in a patient born with absence of the hormone. Subjects will be closely monitored to avoid complications.
- g. This study involves overnight hospitalization, at considerable inconvenience, involving even the parents who must accompany their volunteer children to the hospital for admission and discharge.

### **2. Procedures for Minimizing Potential Risk**

The repetitive blood sampling procedures will be performed after the patient is admitted to the General Clinical Research Center of the University of Chicago Hospitals to the



Pediatric Endocrinology (General Pediatric) Service, with a research nurse with pediatric experience in constant attendance to carry out the repetitive blood sampling and monitor patients closely, a resident in pediatrics available in-house, and the Pediatric Endocrinology service on call. Vital signs are taken frequently throughout the procedures. Blood volumes will be adjusted so that no more than 5% of blood volume will be removed in 24 hr, 10% in a month. Iron stores will be replenished by prescribing ferrous sulfate.

### **3. Potential Benefits and Risk/Benefit Explanation**

Patients with disorders of puberty benefit from the diagnostic accuracy of the sleep test for the subtle hormonal changes of early puberty, as well as the interpretation of the results of GnRHag test.

Factrel® (GnRH itself) is the standard diagnostic agent used by pediatric endocrinologists to attempt to distinguish among disorders of puberty. However, there is often not an available alternative to the GnRHag test because the Factrel supply is erratic; like so many marginally profitable drugs, this drug is often unavailable at the time of need for prompt diagnosis.

There is no direct benefit to volunteers. However, this study is potentially beneficial to society by improving diagnostic accuracy of disorders of puberty so that they can be more quickly and specifically treated. There is a pressing need for sex-, age- and pubertal stage-specific normative data for use in determining what is normal and what is abnormal.

### **K. Monitoring of Safety of Subjects**

Safety monitoring will be conducted annually by Drs. Rosenfield, Baumann, and Radovick via data review. In addition, ongoing monitoring will be done as the patient goes through the protocol by the GCRC nursing staff and the Pediatric Endocrinologist on call. The PI will grade adverse events according to the 0-5 scale shown in the DSM guidelines. The PI will determine the relationship of adverse events to the test procedure using the scale in the DSM guidelines. Adverse events will be reported in writing to the IRB, the GCRC, and the FDA. Treatment will be stopped in the event of severe allergic reaction or if IV access is not possible to achieve.

### **L. Payment to Subjects:**

Patients will not be paid for participating in the study. Healthy volunteers will be compensated \$50 for completion of the sleep test, \$100 for completion of the GnRHag test, or \$150 for completing both the sleep test and the GnRHag test. Volunteers will also be given one parking sticker, allowing free parking, for each day they are in the Hospital during the study. Volunteer children will be reimbursed by check in their own name.

### **M. Procedures to Obtain and Record Informed Consent:**

The principal investigator, co-investigator, or M.D. or R.N. associate will obtain written consent prior to any procedures or tests are initiated after informing subjects fully about the project, procedures associated with it, and all experimental procedures. The nature and purpose of the study and the risks and benefits of participating in the study will be explained in detail to the patient and family. Possible risks, discomfort and their consequences will be explained and the subjects will be told that they may withdraw their consent at any time and that such withdrawal will not restrict access to health care services at the University of Chicago Hospitals. The subject will have the opportunity to ask questions concerning any and all aspects of the

project and any procedures involved. Special attention will be given to ensure that the study is explained to the patient at a level that he or she can understand. In order to assure that the patient understands the information given, the doctor or nurse obtaining the consent will ask that the patient explain the study in his or her own words back to the person obtaining consent. The patient will specifically be asked whether he or she would like to participate in the study. All procedures associated with the study will be explained and answers to any questions or concerns will be given. Any patient declining participation will not be included in the study, whether or not the parents want him or her to participate. A copy of the signed consent and assent forms that explain the study will be given to the patients and families for their record. Phone numbers for research personnel and the IRB will be included in the consent in the event that patients have questions or concerns at a later date. No guarantee or assurance as to the results to be obtained will be given.

**N. Procedures to Maintain Confidentiality of Research and Study Subject Materials:**

DNA samples may be sent to an outside laboratory, yet to be determined, for genetic testing not available at the University of Chicago. However, to protect the subject's identity, these blood samples and test results will be identified only by ID number. The record linking the ID number to his or her identity will be kept under lock and key in the Clinical Research Center and the offices of Dr. Rosenfield, and only he and his research staff will have access to this record.

Data from this study may be used in medical publications or presentations. The subject's name and other identifying information will be removed before this data is used. If we wish to use identifying information in publications, we will ask for the approval of the subject or his or her legal guardian at that time.

**O. Bibliographic References:**

See enclosed research protocol.

**P. Recruiting Methods:**

Healthy volunteers will be recruited through the use of ads posted throughout the University of Chicago Hospitals and University Campus. We will also recruit volunteers via ads placed in various newspapers and publications. Responses to the ads will be addressed by the Pediatric Endocrinology Research Nurses.

Patients with disorders of puberty will be recruited from the Pediatric Endocrinology Clinic by their personal endocrinologist.

**Q. Description of How the Subject's Primary Physician will be Notified of and Involved in the Proposed Research:**

The primary physician receives a letter from the attending pediatric endocrinologist summarizing the history, physical and treatment recommendations and goals for patients seen in the Pediatric Endocrinology Clinic at the University of Chicago Hospitals. The primary physician also receives a copy of the letter sent to parents of the patient describing the same topics. Through this letter, the primary physician is made aware that the study was presented to the patient and his or her family as an option.

After the study results are back, the Principal Investigator informs the referring endocrinologist of the results so that he or she can inform the family. Patients of the investigators are sent a letter, as is their primary physician, explaining the results.

**R. Description of Anticipated Coordination Between Interdepartmental Faculty:**

Not applicable.

**S. How and When Pregnancy Tests Are Performed:**

Not applicable because patients are at most early pubertal.

**T. Rationale for Excluding Women, Minorities and/or Children:**

Not applicable, since there are no such exclusions.

**U. Drug Infusion Procedure:**

No drug infusions will take place with this protocol. However, leuprolide acetate will be given subcutaneously on the morning of Day 2 by trained, registered nurses in the GCRC.