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

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GnRH Agonist Test in Disorders of Puberty. Outline.

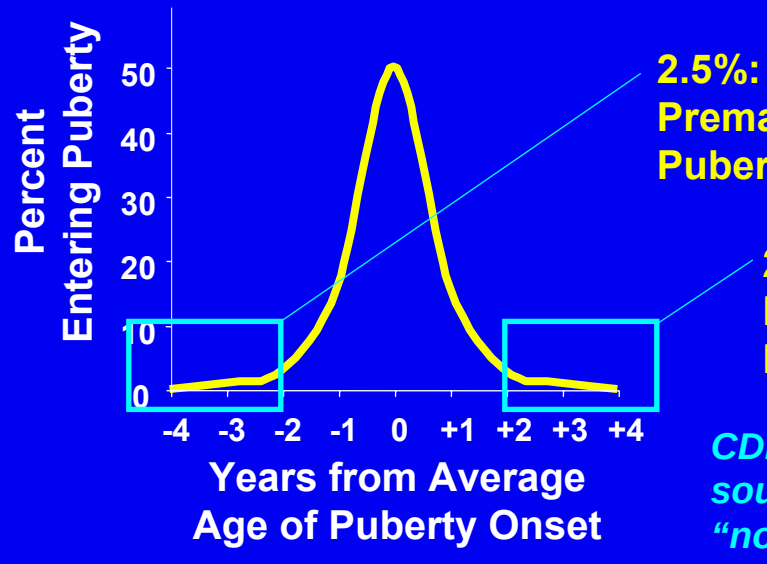
- Overview
- The nature of the problem
- Background endocrinology
- Background of this study: antecedent studies
- Adverse events of leuprolide
- Protocol #13472A
- Summary

Overview:
GnRH Agonists are Promising Diagnostics

- Delayed Puberty (esp. a diagnostic problem in boys)
  - Constitutional Delay of Puberty (CDP, *"an extreme variant of normal"*) vs
  - Gonadotropin Deficiency (GnD)
- Premature (Precocious) Puberty (esp. in girls)
  - Idiopathic True / Central Precocious Puberty (CPP, *"an extreme variant of normal"*) vs
  - Normal Prepubertal *and*
  - Premature Pseudo-Puberty (diverse types)
- Need for Normative Data on Healthy Prepubertal & Early Pubertal Children

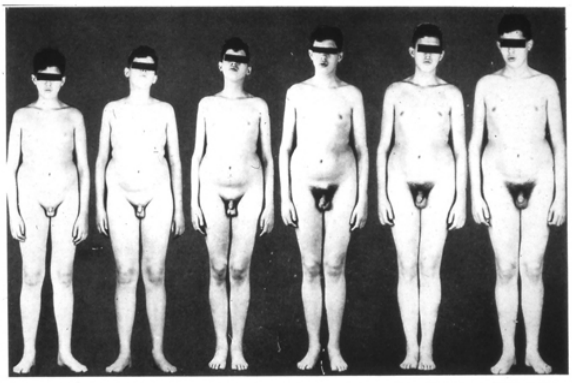
CDP & Idiopathic CPP: *"Extreme Variants of Normal"*

Conceptual Definition of Premature & Delayed Puberty



CDP and CPP are source of most "normal" data

Problem 1: Differentiating Constitutional Delay of Puberty (CDP) from Gonadotropin Deficiency (GnD)



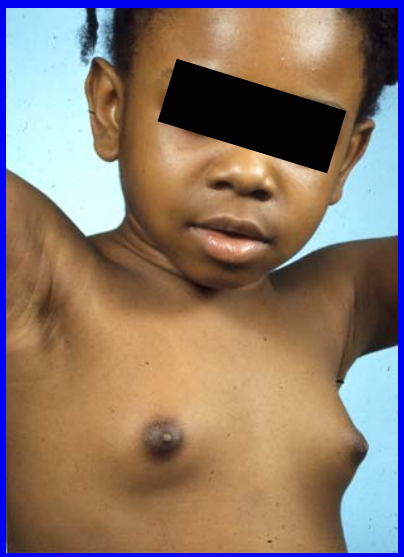
17.3 18.0 18.7 19.5 20.3 21.8

Longitudinal F/U of Boy with CDP (years)

Wilkins L: The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence. Thomas, Springfield, 1968.

- Delay mostly in boys
- CDP boys develop increasingly poor self-image after 14 years
- "Grow out of it"
- Cause: "nl variant"
- W/U: minimal
- Rx: reassurance ± 6 month T boost
- Contrasts with GnD

Problem 2: Differentiating Idiopathic CPP from Normal Variants & Other Pseudo-Precocity. I.



- Precocity predominantly in girls
- CPP scary for child & parents
  - » moody
  - » periods?
  - » early growth arrest
- Over > 90% "nl stage just early"
- W/U: minimal (brain MRI when rapidly progressive)
- Rx: reassurance ± GnRH ag chronically until ~11 yo
- Pseudo-precocity may be normal variant or due to neoplasm, etc

Problem 2: Differentiating Idiopathic CPP from Normal Variants. II. The Problem of Early Thelarche

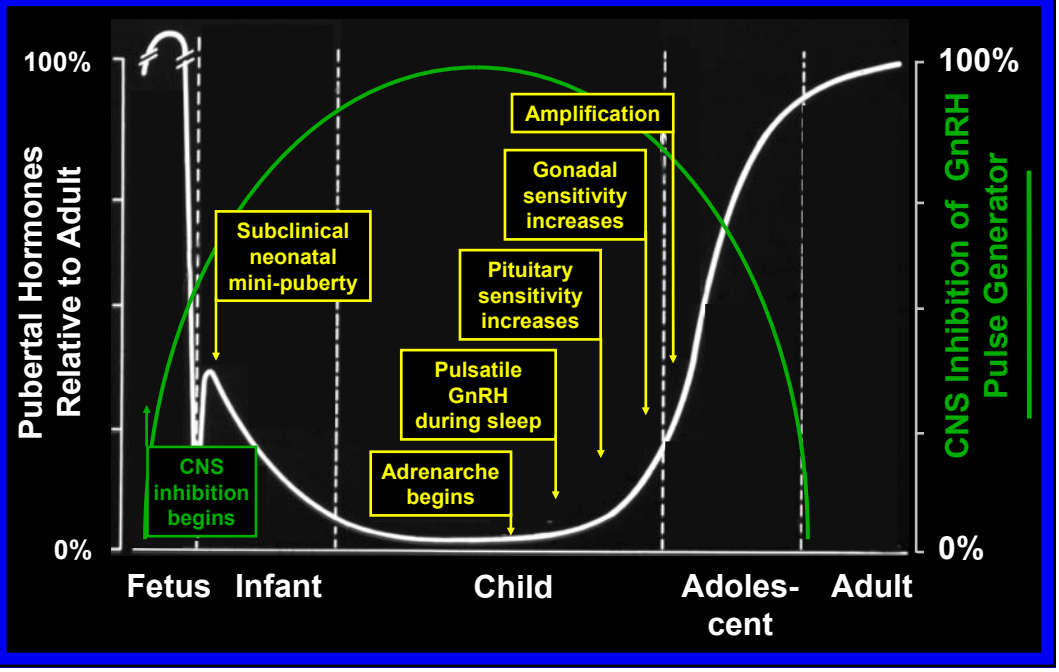
"Yesterday's Precocious Puberty is Norm Today"
- N Y Times, December 7, 1999

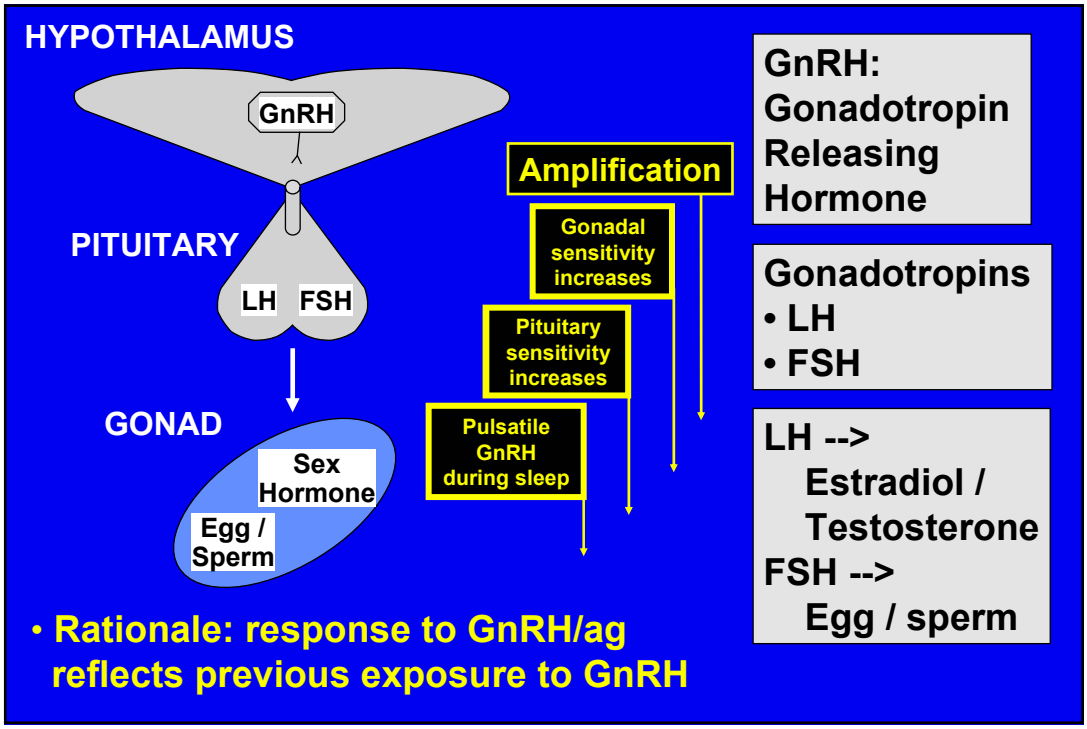
"Doubters Fault Theory Finding Earlier Puberty"
- N Y Times, February 20, 2001

"2 Endocrinology Groups Raise Doubt on Earlier Onset of Girls' Puberty"
- N Y Times, March 3, 2001

- *While there is some evidence that breast development may be occurring 1-2 years earlier, esp. in the obese, age of menarche is unchanged--is this true puberty?*

BACKGROUND ENDOCRINOLOGY OF PUBERTY





BACKGROUND OF GnRH AGONIST TESTING. I.

- 1977: Nobel Prize for Discovery of GnRH
- '80's: Desensitizing Effect of Chronic GnRH Agonist Analogs -> Chronic GnRH Agonist Rx for CPP (ODP)
- 1985: PI GCRC Studies Under Expanded Syntex IND for Nafarelin Treatment of Central Precocious Puberty
  - Examined patients' hormonal responses to 1st dose of nafarelin (out of my interest in the acute response being a potentially useful diagnostic test)

Endogenous GnRH (Factrel®)

1 2 3 4 5 6 7 8 9 10

pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2

Nafarelin (Synarel®)

1 2 3 4 5 6 7 8 9 10

pGlu-His-Trp-Ser-Tyr-NH-CH-CO-Leu-Arg-Pro-Gly-NH_2

CH_2

Leuprolide acetate (Lupron®, generic)

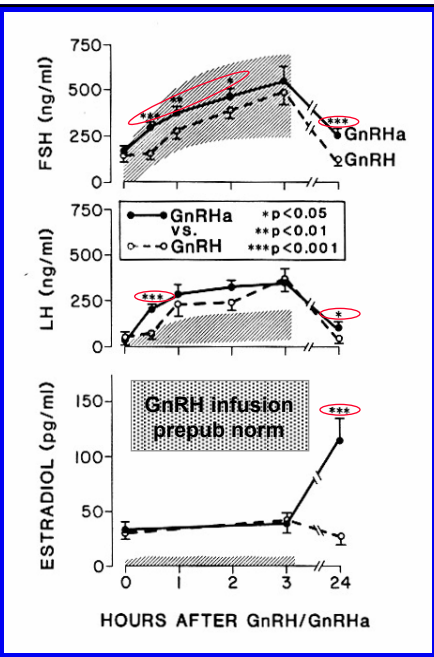
1 2 3 4 5 6 7 8 9 10

pGlu-His-Trp-Ser-Tyr-NH-CH-CO-Leu-Arg-Pro-Gly-NH- Ac

CH_2

OH

- **Subjects: CPP girls starting nafarelin (GnRHa) Rx**
- **Compared GnRH 3 hr infusion test to a nafarelin test of pituitary-gonadal axis**
- **Results: LH & FSH responses to GnRHa greater & more prolonged than to GnRH --> estradiol response**



Rosenfield, et al JCEM 1986

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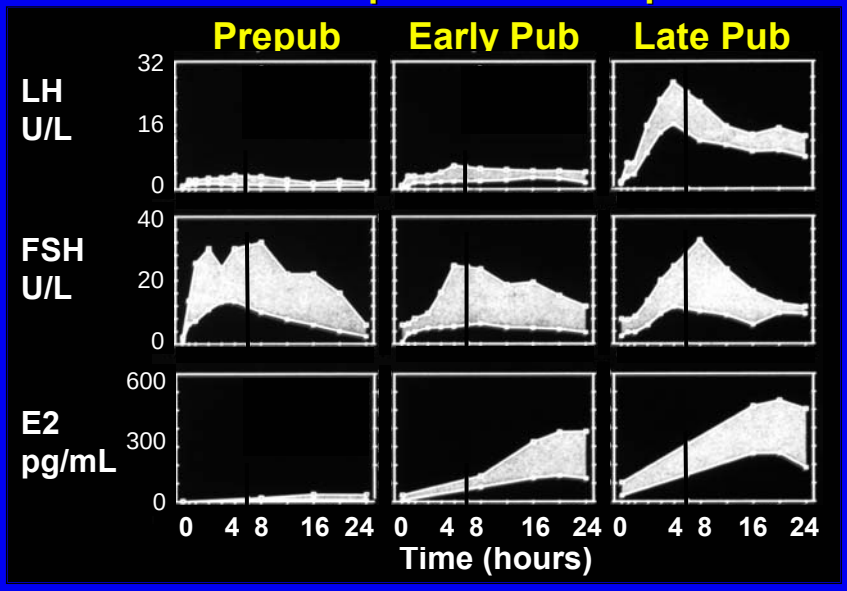
- 1985: PI GCRC Studies Under Expaned Syntex IND for Nafarelin Treatment of Central Precocious Puberty
  - Comparison of CPP's hormonal response to nafarelin dose #1 by injection to natural GnRH infusion test
    - » showed acute agonistic effect on LH-FSH-E2
  - **13472A precursor protocols were pilot studies to explore diagnostic potential of nafarelin in children with known or suspected disorders of puberty (mostly CDP or CPP)**

Girls with variations of normal pubertal development

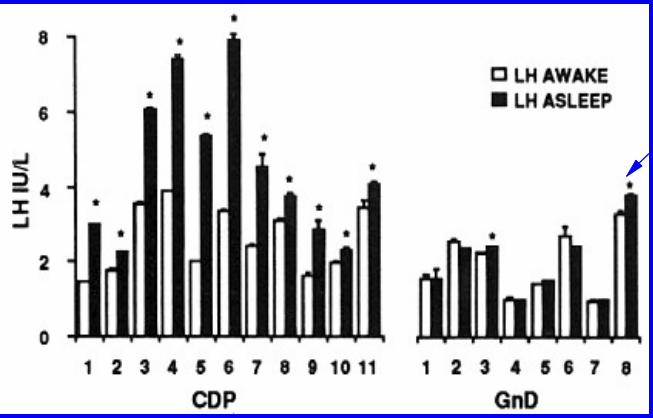
Nafarelin tests in girls at various pubertal stage

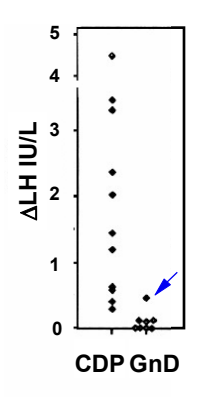


Goodpasture, et al. Clin Obstet Gyn 1993

BACKGROUND: GnD vs CDP in prepubertal boys

Sleep Tests:



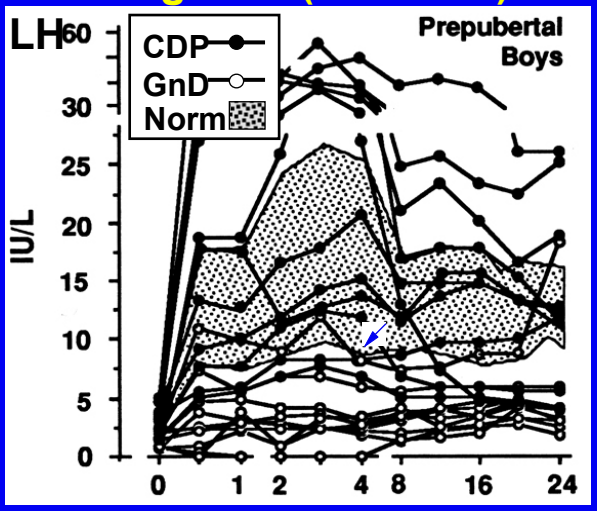


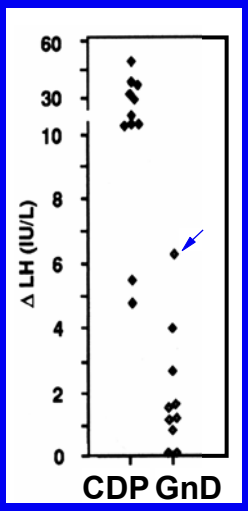
Provisional sleep test discriminatory criterion: ΔLH ≥ 0.35 U/L separates 18/19

Ghai, et al JCEM 1995

BACKGROUND: GnD vs CDP in prepubertal boys

GnRH Agonist (Nafarelin) Tests:





ΔLH ≥ 4.8 U/L discriminates 19/20

Ghai, et al JCEM 1995

BACKGROUND OF GnRH AGONIST TESTING. II.

- 1992-93: Syntex *sold out* & Searle not interested

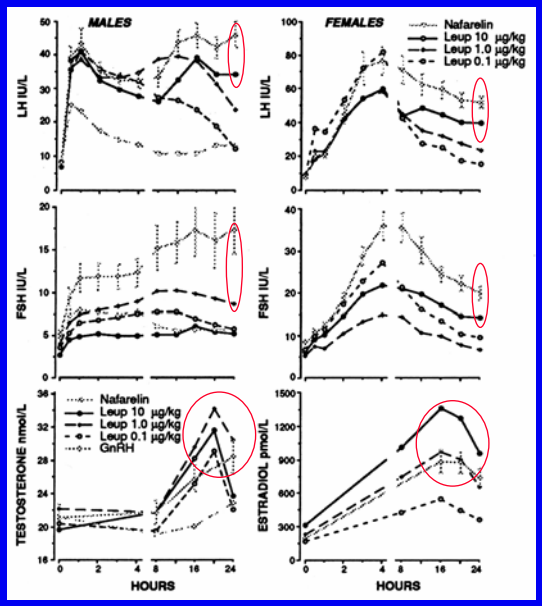
  PI obtained IND #40,387 (1992): '93 nafarelin -> leuprolide
  - Several GCRC protocols with co-investigators
    - » Hyperandrogenism in adult women and children
    - » Disorders of puberty (CDP, CPP, etc)
    - » TAP 1 yr bridge funding --> no further support
  - FD-R-001012 (1994, ODP)
    - » Adult dose-response study & comparison to naf/GnRH
    - » GnD vs CDP
  - FD-R-001473 (1997, OPD)
    - » Adult GnD trial of intermittant GnRHag Rx

  IND #60,003 (2000): leuprolide
  - RO1-HD-39267 ('01): hyperandrogenism (child & adult)

Dose-response study of leuprolide (Lupron®) in adults + comparison to GnRH (Factrel®) in men & historical nafarelin data

Results

- Lupron 10 mcg/kg similar to naf in LH & sex steroid stimulation
- Lupron less potent FSH stimulant



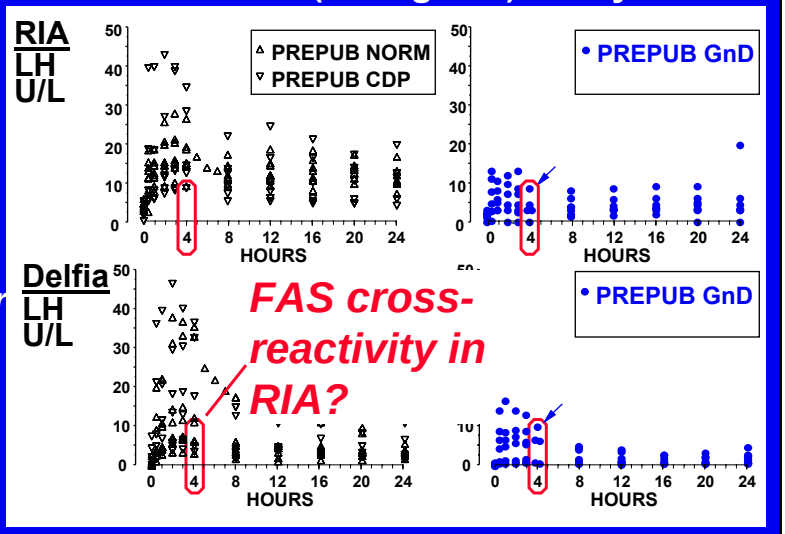
--Rosenfield, et al JCEM 1996

Snags!

1. RIA results unlike monoclonal ("3rd gen.") assay for LH

Why?

- *Microhet-erogeneity of LH*
- *Our RIA had enhanced specificity for bioactive LH, but incomp.*
- *Delfia ß-subunit specific*

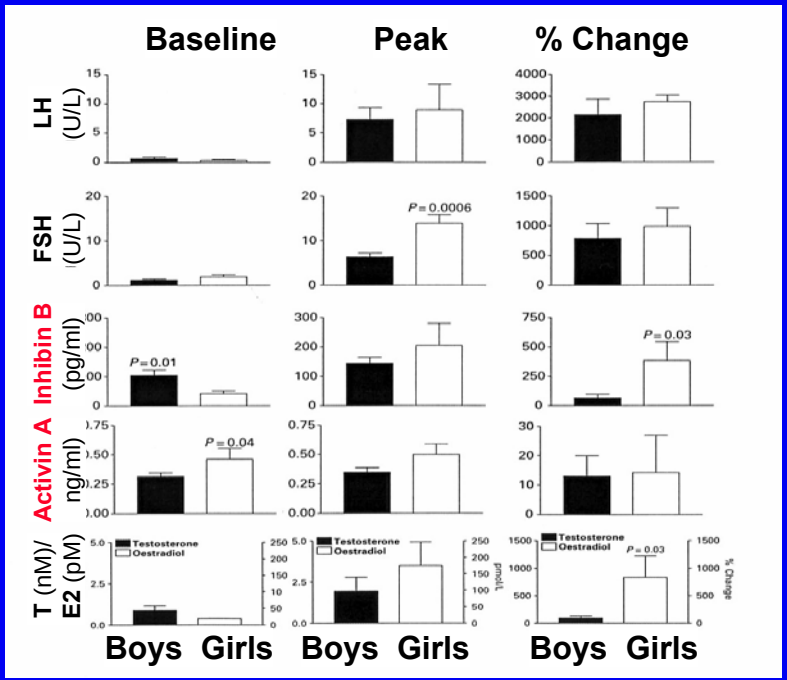


2. Alarmed freezer failure: lost samples before 2001

Meanwhile-- other sex-specific potential end-points discovered in pubertal variants

Boys (n=11) & girls (n=7) w. BA >7.8 y

-- *Elsholz, et al Hum Reprod 2004*



BACKGROUND OF GnRH AGONIST TESTING. III. Summary to Date.

- Leuprolide not quite same as nafarelin re FSH stim
- Can't go back to discriminatory RIA
- Considerable promising preliminary data in children from multiple peer-reviewed studies at many levels (GCRC/site visits, FDA- and R01-reviews)
- Starting over

ADVERSE EVENTS OF LEUPROLIDE TEST (1 Inj x)

Leuprolide tests in 577 adults & children: U of C 1993-2005

- No Serious Adverse Events
- Anticipated Side Effects
  - Children under 18 years of age (n = 332)
    - » IV-related: 3 (soreness, hematoma) -> one withdrew
    - » local allergic reaction (rash), transient: 1
  - Adults (n = 245)
    - » IV-related: 1
    - » local allergic reaction (rash), transient: 1
    - » Hormone-related side effects (post-study): 14
      - – menstrual pattern change: 3 (1 pre-existing PCOS)
      - – PMS symptoms mood, cramps, h/a: 11 (1 of the 3 *males* improved)

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ADVERSE EVENTS OF LEUPROLIDE *TREATMENT*

Wide use of leuprolide long-term Rx (Depot Lupron®)

- Children with CPP (short stature)
- Adult men with prostate cancer
- (Adult women with endometriosis, fibroids, fertility Rx)

Side effects of long-term Rx

- Depot -> 5-10% develop sterile abscesses at injx sites
- Hormone-related side effects
  - » Menstrual irregularity
  - » PMS symptoms (mood changes, swelling, h/a, etc)
  - » Memory effects? (data contradictory--*Yaffe, JAMA 2003)*
  - » osteopenia
- No increase in birth defects *(Jannsens 2000)*

ADVERSE EVENTS OF LEUPROLIDE ACETATE. Response to Adverse Public Comment (4 letters)

Mis-informed and/or related to long-term therapy

- Package insert adverse effects are those of *2 yr Rx of prostate ca*
- *No* "black box" warning
- Human evidence for adverse effect on autoimmunity *insufficient* to warrant warning
- *Not* a hazardous drug requiring chemo precautions

Other Public Comments

- Lawson Wilkins Pediatric Endocrine Society, The Endocrine Society, The American Society for Reproductive Medicine are *un*concerned about leuprolide acetate test toxicity
- LWPES notes that "leuprolide is used in the routine diagnostic testing of children to determine the initiation of puberty…highly useful…normative data are sparse"
- The Endocrine Society *adds* that, while determining sleep-related LH secretion is the "gold standard," it is (potentially) "less invasive than the leuprolide test."

PROTOCOL 13472A:
GnRH Agonist Test in Disorders of Puberty

Hypothesis

Hormonal responses to GnRH agonist (GnRHag) test will distinguish among disorders of puberty as well as a sleep test.

Specific Aims

1. Distinguish among the causes of premature puberty:
   a. idiopathic CPP *(vs. prem. thelarche)* vs healthy vols
   b. gonadotropin-indep precocity (e.g., tumor) vs idiopathic CPP
2. Distinguish among the causes of delayed puberty:
   a. GnD vs CDP

CDP & Idiopathic CPP: *"Extreme Variants of Normal"?*

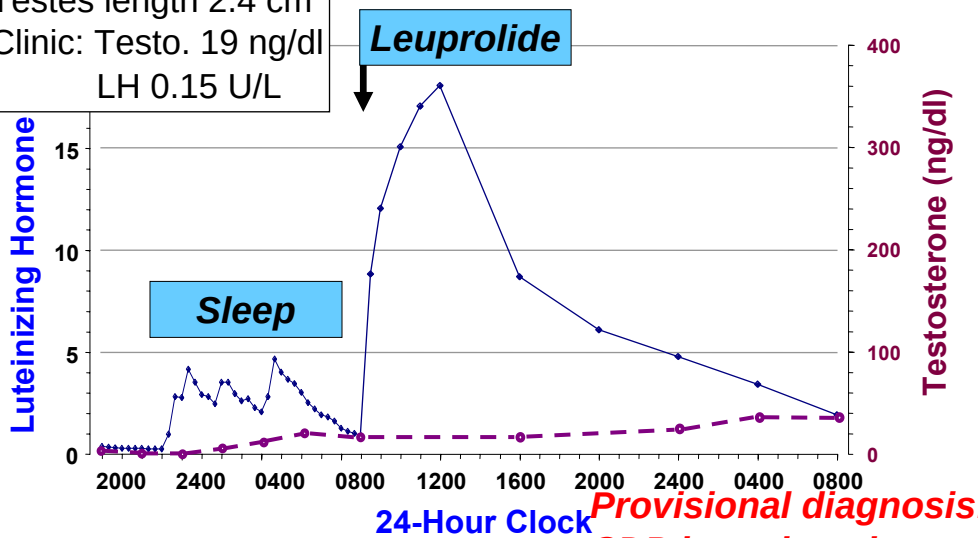
- Practice assumes that these are normal variants
  - Pubertal tempo, menstrual cyclicity, and fertility in adult life typically within broad range of normal
  - Familial in about half
- Evidence that a small percent of these "normal variants" may *not* be normal:
  - Slow tempo of those starting puberty at 6-7 years
  - Family history of delayed puberty in ~10-15% of GnD
  - GnRH receptor SNPs nominally associated with variations in timing of puberty *(Sedlmeyer, JCEM Oct '05)*
  - Mouse chromosomes 6 and 11 harbor genes that regulate pubertal timing *(Krewson,Endocrinol 2004)*
- Normal population data needed to avoid misclassif.

PROTOCOL #13472A: GnRH Agonist Test in Disorders of Puberty

Study Design

- **Subjects: 20 per group of each sex.**
  - » **Normal volunteers:**
    - – **Prepubertal male, 9-13 years old**
    - – **Prepubertal female, 8-12 years old**
    - – **Early pubertal male, 9-15 years old**
    - – **Early pubertal female, premenarcheal, 9-15 years old**
  - » **Patient groups**
    - – **CDP vs GnD**
    - – **CPP vs gonadotropin-independent precocity and premature thelarche**

16 year old boy
Prepubertal, short
Testes length 2.4 cm
Clinic: Testo. 19 ng/dl
LH 0.15 U/L



Provisional diagnosis: CDP in early puberty

GnRH Agonist Test in Disorders of Puberty

Analysis of Data. I.

Sleep test: significant increase LH ≥ 0.35 U/L provisionally defines puberty onset

- Normal range set at 5-95 %ile healthy volunteers
  - » Secondary: 5-95%ile for CDP (boys) & CPP (girls)

GnRH agonist (leuprolide) test:

- Hormone response primary variables (group-specific)
  - » Pituitary: LH, free alpha subunit (FAS)
  - » Gonads: Sex steroid (T or E2), inhibin-B
- Sex- and stage-specific 5-95%ile ranges set for:
  - » Normals (healthy volunteers)
  - » CDP and CPP

GnRH Agonist Test in Disorders of Puberty

Analysis of Data. II.

- Boys *(common dx problem is delayed puberty)*:
  - » Primary comparison: GnD vs CDP (*stage-specific*)
  - » Secondary: GnD vs healthy volunteers *and* CDP vs healthy volunteers
  - » *Tertiary: girls**
- Girls *(common dx problem is premature puberty)*:
  - » Primary: CPP vs *pre*pubertal healthy volunteers
  - » Secondary: CPP vs *pseudo-pubertal* groups* (gonadotropin-independent precocity and premature thelarche)
  - » *Tertiary: boys**

* *Power is limited for some sub-groups*

GnRH Agonist Test in Disorders of Puberty: Summary

- GnRH agonist testing is of minimal risk
- Study design straight-forward: normal vs abnormal
- Adequate statistical power for primary comparisons
- Significance for clinical care: great
  - » This protocol will develop badly needed data on the hormonal responses to leuprolide in normal prepubertal and pubertal children, using commercially available state-of-the-art assays
  - » It will also provide data on the diagnostic value of the test for the most common pubertal disorders