FSH Glycoforms: Diagnostic and Therapeutic Potential for Women

Jeff May, Ph.D.
Research Scientist
Dept. Biological Sciences

May 9, 2018
Aging Pituitary-Gonadal Axis

P01 6AG029531, 5 year grant, ~$10.6 Million
PI – G. Bousfield
National Institute on Aging/National Institutes of Health

Organizational Chart

EAC
James Dias, Ph.D.
Kelly Mayo, Ph.D.
Alfredo Ulloa-Aquirre, Ph.D.

P01- Core A
George Bousfield, Jeffrey May

Administrative Assistant
Yonai

Project 1
Bousfield, WSU

Project 2
Davis, UNMC

Project 3
Kumar, U Col. MC

Core B
May, Butnev, Shuai

Core C
Guda, UNMC

IAC
George Bousfield, Ph.D.
Jeffrey May, Ph.D.
William Hendry, Ph.D.
Howard S. Fox, MD, PhD

Wichita State University
Follicle-Stimulating Hormone (FSH) - Capsule Summary

1. Heterodimeric glycoprotein, mw \( \approx 30,000 \) (2 subunits with carbohydrate)

2. Produced by gonadotrophs of the anterior pituitary, crucial for female reproduction

3. Regulation in the female:
   - GnRH and Activin - positive
   - Inhibin and Estrogen - negative

4. Ovarian functions include [among many]:
   - Steroidogenesis (estradiol-17β)
   - LH receptor induction, oocyte maturation, GC proliferation
   - Antrum formation, gap junction formation
Follicle-Stimulating Hormone (FSH)
FSH for clinical use was originally isolated from the urine of menopausal women.
Age-Related Changes in FSH Glycoform Abundance in Individual Human Pituitaries

![Graph showing relative abundance of tetra- and di-glycosylated forms of FSH in different sources.](image)

[Pituitaries provided by Dr. Naomi Rance., Univ. Arizona]
Fully glycosylated hFSH24

“Classic” FSH24

Hypo-glycosylated hFSH21/18
Thus, hFSH Glycoform Relative Abundance Changes with Age

So What!!!!!
Obtained Rat Pituitary Somatotrophs (GH3 cells) transfected with the human FSH α and β subunit genes. Grew cells in large scale culture to produce *milligram* quantities of FSH to test glycoform bioactivities.

Can isolate hFSH glycoforms from pituitaries but they are hard to get and yields are low!!
Conditioned medium from the GH3 cells
Undergoes complicated purification regimen
To produce hFSH24 and hFSH21/18
Comparison of FSH$^{21/18}$ Versus FSH$^{24}$

1. Association rate of FSH21/18 to the ovarian FSH-Receptor is much faster than FSH24

2. FSH21/18 binds to the receptor with higher affinity than FSH24

3. In virtually every ovarian bioassay tested to date, FSH21/18 is 3-10-fold more potent than FSH24 (steroidogenesis, LH receptor, induction, cAMP and MAPK activation, etc.)

**FSH21/18 seems to be more bioactive than FSH24!!**
However, FSH21/18 abundance declines with age!!

FSH  
21/18 24

Young  High  Low

Old  Low  High
Summary: What NOW Do We Know About hFSH! (and how can we leverage this into clinical utility?)

- Critical for female fertility and the production of estrogen
- Exists as glycoform variants due to differences in the number of glycans (CHO)
- Glycoforms can be purified, separated, isolated, and measured
- The relative abundance of the glycoforms changes with age in women
- The glycoforms exhibit marked differences in in vitro bioactivity
Potential Clinical Applications

1. **Diagnostic** – can our knowledge of hFSH glycoforms tell us something about the health status of a woman?

2. **Therapeutic** – Can our knowledge of hFSH glycoforms be used to treat a health condition?

3. **Disease Prevention/Amelioration** – Can our knowledge of hFSH glycoforms be used to mitigate a disease/physiological state
1. Diagnostic Potential:

Goal: Determine hFSH24 and hFSH21/18 relative levels during the menstrual cycle and as a function of age via urinary analysis.

Urine collection and neutralization
Ethanol precipitation
Centrifugation, re-suspension of pellet
Aqueous solubilization
Immunoaffinity isolation of hFSH
Lyophilization
Analysis via Western Blot

hFSH21/18 and FSH24 can be separated by SDS-PAGE and identified by immunoadsorption using anti-FSHβ antibodies
Develop monoclonal antibodies specific to hFSH24 and hFSH21/18

6 Ag ELISA of Important Monoclonals

- CTRL

+ CTRL

15-1 E3 D7 (anti-β)

17-6 E5 A4 (anti-α)

10-9 C11 C10 (anti-α)

14-2 G5 H4 (anti-β)
Identify and produce mAbs against hFSH24 and hFSH21/18 for use in ELISAs or RIAs to quantitate glycoform levels and eventually

Seek to develop a urinary-based, self-assessment tool for women to monitor hFSH glycoform status, perhaps via a “dip-stick” mechanism.
Diagnostic Potential of hFSH Glycoform Assessment:

Can it be utilized to assess the “normalcy” of a reproductive cycle

Can it be used to monitor onset of the peri-menopause

Is hFSH glycoform variance associated with various types of infertility:
    Unexplained
    PCOS
    Poor responders
**Diagnostic** – can our knowledge of FSH used to predict the onset of the peri-menopause??

Menopause: cessation of menstrual activity (climacteric) loss of a women’s natural fertility demise of the ovarian reserve of germ cells (oocytes) loss of estrogen (osteoporosis, hot flashes, cardiovascular health, weight changes, etc.) mean age = 51 years

Peri-Menopause starts 10-12 years prior to the menopause associated with subfertility/infertility irregular menstrual cycles (number/duration/intensity) decreasing estrogen (?) clinical markers = cycle day 3 FSH (↑) and AMH levels (anti-mullerian hormone, ↓)
Glycoform Abundance Changes With Age!!

FSH
21/18  24
Young     High      Low
Old       Low      High

Can the change in the ratio of FSH\textsubscript{21/24} predict the onset of the peri-menopause???
Bone loss increases during the peri-menopause but increases markedly at menopause.
Which will provide the earliest marker of pituitary/gonadal axis aging in women, the ovary or the pituitary???
2. **Therapeutic** – Can our knowledge of hFSH glycoforms be used to *treat* a health condition?

*How about Infertility!!!*

Recombinant hFSH is used extensively in assisted reproduction.
Problem: The ovary becomes increasingly resistant to stimulation with age!

With increasing age, one needs more drug given over a longer period and you still get fewer oocytes!!
However, FSH preparations used clinically are largely FSH$_{24}$.!!!
Recombinant FSH produced for use clinically is largely FSH24!!!
In virtually every in vitro bioassay we have tested to date using rat, pig, and human models, FSH$_{21}$ is consistently more potent than FSH$_{24}$ (5-26 fold):

- receptor binding assays
- steroid production
- induction of LH receptors

So, it would appear that clinically, a less active form of FSH is being used to stimulate an increasingly resistant ovary!!

Could hFSH$_{21}$ be more effective??
While provocative, the *in vitro* results do not prove the effectiveness of FSH$_{21}$ versus FSH$_{24}$ *in vivo***!!

As a start, studies are needed in animals models to test proof of principle prior to testing in humans!!

Have submitted a Multi-PI R01 NIH entitled “FSH Glycoforms and Ovarian Function” To undertake IVF comparing hFSH24 and hFSH21/18 in a non-human primate Model
3. Disease Prevention/Amelioration

Can our knowledge of hFSH be used to mitigate a disease/physiological state?

**How about osteoporosis!!!**

- represents a *major* public health concern (group of etiologies marked by a reduction in bone mass relative to bone volume)

- over time dramatically weakens bones increasing the risk of fractures involving the spine (46.6%), hip (20%), wrist (16.6%), and other assorted sites (20%).
Landmark Paper:

Using transgenics, they knocked out the FSHβ gene in mice

Sooooo,

Mice couldn’t make functional FSH and hence, couldn’t make estrogen!
Resulting hypo-estrogenic mice should have exhibited accelerated bone loss!

They did not, hence, something other than lack of estrogen must be contributing to bone loss!!
Perhaps FSH??????
Took pre-osteoclast cell line (RAW267.4), treated with RANK-L alone or with FSH$_{21}$ or FSH$_{24}$, and looked at the induction of tartrate-resistant acid phosphatase (TRAP), a positive indicator of osteoclast differentiation.

Thus, FSH$_{24}$ but not FSH$_{21/18}$ stimulated osteoclast differentiation.

Osteoclasts degrade bone!
<table>
<thead>
<tr>
<th></th>
<th>Levels in Women</th>
<th></th>
<th>Bioactivity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young</td>
<td>Old</td>
<td>Ovary</td>
<td>Bone</td>
</tr>
<tr>
<td>hFSH$_{21}$</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>hFSH$_{24}$</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>

FSH glycoform dominance seems to change from that which is ovary active in young women to that which is bone resorptive in older women.
Clinical Impact of FSH Suppression Upon Bone Loss in Menopausal Women – In Revision

PI - Jeffrey V. May, Ph.D.
Grant # = 1R01 AG038478-01
TDC = $3,045,854

Dual-Site, 27 Month, Interventional Clinical Trial
(Wichita & Omaha, NE)

Wichita State University
Univ. Kansas Medical Center-Wichita
Osteoporosis Research Center (Omaha, NE)
Clinical Research Inst (Wichita)
Wichita Radiology Group
Affiliated Medical Services (Via Christi MC, Wichita)

4-year Project, 112 Subjects, 56 per site
FSH Glycoforms: Diagnostic and Therapeutic Potential for Women

Considerations for Clinical Application

1. **Diagnostic** – can our knowledge of hFSH glycoforms tell us something about the health status of a patient?

2. **Therapeutic** – Can our knowledge of hFSH glycoforms be used to treat a health condition?

3. **Disease Prevention/Amelioration** – Can our knowledge of hFSH glycoforms be used to mitigate a disease/physiological state
Questions????