We do need LH/hCG, and administration regimens could be critical

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Science has proof without any certainty
Creationists have certainty without any proof

Ashley Montague
*British Anthropologist*
*(1905-1999)*
How to provide LH activity

- human-derived LH
- recombinant LH
- human CG
  - conversion factor
    1 IU hCG = 6-8 IU LH
- recombinant CG
  - conversion factor
    1 µg rCG = 25-30 IU hCG

<table>
<thead>
<tr>
<th></th>
<th>LH content</th>
<th>CG content</th>
</tr>
</thead>
<tbody>
<tr>
<td>hMG</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>HP hMG</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>rLH</td>
<td>+++</td>
<td>0</td>
</tr>
<tr>
<td>hCG</td>
<td>0</td>
<td>+++</td>
</tr>
<tr>
<td>rCG</td>
<td>0</td>
<td>+++</td>
</tr>
</tbody>
</table>
The 2-cell, 2-gonadotropin concept:
A model revisited

- Theca
- FSH
- Granulosa
- Aromatase
- Proliferation
- LH
- Mid-late FP
- Follicle growth
- Preantral and small antral follicles (<10-12 mm)
  - Stimulation of **theca cell** androgen production

- Large antral follicles (>10-12 mm)
  - Stimulation of **theca cell** androgen production

**FSH-like actions**
- Stimulation of **granulosa cell** proliferation and growth
- Induction of **granulosa cell** aromatase to catalyze estrogen formation
Potential clinical implications of LH/hCG receptor expression by ovarian granulosa cells

Due to mid-late follicular phase interaction with granulosa cells, LH activity can:

- increase estrogen synthesis
  - enhanced availability of theca-derived androgen substrate
  - stimulation of aromatase
- modulate folliculogenesis
- synergize with FSH
- replace FSH
Rationale for LH activity supplementation

- estrogen effects on oocyte
- modulation of folliculogenesis
- FSH synergies
- FSH replacement
Serum levels of gonadal steroids
(Kilani et al, Hum Reprod, 18:1194, 2003)

- **$E_2$ (pg/mL)**
  - $P <0.05$

- **$P$ (ng/mL)**
  - $P$ NS

- **$T$ (ng/mL)**
  - $P$ NS

**preovulatory $E_2$ (pg/mL)**
- $P <0.005$

**preovulatory $P$ (ng/mL)**
- $P$ NS

**preovulatory $T$ (ng/mL)**
- $P$ NS

Graphs show the changes in $E_2$, $P$, and $T$ levels over 10 days of treatment with rFSHα and HP hMG.
Estrogen mechanisms - oocyte

- estrogen receptors are present in human oocytes
  - Wu et al, Fertil Steril 59:54, 1993

- use of antiestrogens and of steroid synthesis blockers negatively affected oocyte cytoplasm (not nucleus) maturation and blastocyst development; effects were reversed by E$_2$
  - Yoshimura et al, Biol Reprod 35:943, 1986
  - Yoshimura et al, Endocrinology 120:2555, 1987

- aromatase inhibitors and steroid synthesis blockers lowered E$_2$ and reduced oocyte fertilizability
  - Hibbert et al, PNAS 93:1897, 1996
“Experiments of nature” models

- **17α-hydroxylase deficiency**
  - Rabinovici et al, JCE&M 68:693, 1989

- **17,20-desmolase deficiency**

- **in these conditions:**
  - very low serum and follicular fluid $E_2$ levels are present
  - *in-vitro* fertilization and embryo cleavage were obtained but no development beyond 7-cell stage (Rabinovici et al) nor pregnancy after embryo transfer (Pellicer et al) ensued
Estrogens and oocyte maturation

**summary**

- Ovarian follicle development can progress during FSH-only stimulation and in hypoestrogenic conditions.
- Estrogens do not affect oocyte nucleus and meiotic maturation.
- Oocyte cytoplasm and oolemma maturation are promoted by estrogens.
- Estrogens optimize oocyte fertilization and embryo viability.
Rationale for LH activity supplementation

- estrogen effects on oocyte
- modulation of folliculogenesis
- FSH synergies
- FSH replacement
Follicle development during ovulation induction with HP FSH (150 IU/day) alone or HP FSH (150 IU/day) & hCG (50 IU/day)

Filicori et al
JCE&M
84:2659,1999
Follicle development during ovulation induction with **HP FSH** (150 IU/day) or **hMG** (150 IU/day)

*Filicori et al, JCE&M 86:337, 2001*
Occurrence of small follicles just before ovulation vs. FSH & LH activity administered

*Filicori et al, Hum Reprod 17:2009, 2002*
Rationale for LH activity supplementation

- estrogen effects on oocyte
- modulation of folliculogenesis
- FSH synergies
- FSH replacement
Effects of rLH dose in rFSH treated HH women: preovulatory follicles and E$_2$ levels

European rLH group
JCE&M 83:1507, 1998
Ovulation induction in a patient with hypogonadotrophic secondary amenorrhea

**cycle A**  
(HP FSH)

**cycle B**  
(HP FSH & hCG)

Filicori et al, Fertil Steril, 72:1118, 1999
# Recombinant LH administration in ovulation induction and COS

<table>
<thead>
<tr>
<th>Study</th>
<th>rLH dose (IU/day)</th>
<th>regimen</th>
<th>effects of rLH</th>
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</thead>
<tbody>
<tr>
<td>Sills, 1999</td>
<td>75</td>
<td>COS</td>
<td>none</td>
</tr>
<tr>
<td>Marrs, 2004</td>
<td>150</td>
<td>COS</td>
<td>more transferred embryos</td>
</tr>
<tr>
<td>Acevedo, 2004</td>
<td>75</td>
<td>oocyte donors</td>
<td>more MII oocytes, fertilization &amp; implantation rates</td>
</tr>
<tr>
<td>Humaidan, 2004</td>
<td>rFSH:rLH 2:1</td>
<td>COS from day 8</td>
<td>none</td>
</tr>
<tr>
<td>De Placido, 2005</td>
<td>150</td>
<td>COS from day 8</td>
<td>rLH more effective than rFSH increments in poor responders</td>
</tr>
<tr>
<td>Hugues, 2005</td>
<td>150-1325</td>
<td>OI in PCOS</td>
<td>reduces small follicles</td>
</tr>
<tr>
<td>Lisi, 2005</td>
<td>37.5-75</td>
<td>COS</td>
<td>higher implantation &amp; pregnancy rates</td>
</tr>
<tr>
<td>Fabregues, 2006</td>
<td>150</td>
<td>COS</td>
<td>none</td>
</tr>
</tbody>
</table>
## Gonadotropin dose administered (ampoules)

*FSH only vs. FSH & LH activity in COS*

<table>
<thead>
<tr>
<th>regimen</th>
<th>FSH alone</th>
<th>FSH &amp; LH activity</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Filicori et al, JCE&amp;M 1999</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP FSH vs. HP FSH &amp; hCG</td>
<td>35.6±2.2</td>
<td>23.0±1.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Filicori et al, JCE&amp;M 2001</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP FSH vs. hMG</td>
<td>33.6±2.4</td>
<td>23.6±1.1</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td><strong>Filicori et al, Fertil Steril 2003</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rFSH vs. hMG</td>
<td>25.3±1.3</td>
<td>21.7±0.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Kilani &amp; Filicori, Hum Reprod 2003</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rFSH vs. HP hMG</td>
<td>27.0±1.5</td>
<td>22.4±1.0</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Treatment duration and gonadotropin dose employed

(Kilani et al, Hum Reprod, 18:1194, 2003)

- Treatment duration (days)
  - 8
  - 9
  - 10
  - 11
  - 12
  - 13
  - 14

- Gonadotropin dose (ampoules)
  - 16
  - 18
  - 20
  - 22
  - 24
  - 26
  - 28
  - 30

* P<0.01

- P<0.01
Serum levels of gonadal steroids in hMG vs. rFSH cycles
(Filicori et al, Fertil Steril, 80:390, 2003)

- $E_2$ (pg/mL)
- $P$ (ng/mL)
- $T$ (ng/mL)
Correlations between progesterone levels (AUC) and gonadotropin activity administered

Filicori et al, Hum Reprod 17:2009, 2002
Relationship between premature luteinization and FSH dose and preovulatory LH level during ovulation induction

Bosch et al
Fertil Steril 80:1444, 2003
Endocrine profiles of HP hMG vs. rFSH in 731 cycles
(Smitz et al, Hum Reprod, 22:676, 2007)

- **serum during treatment**
  - in HP hMG cycles higher levels of
    - androstenedione, total testosterone, free androgen index

- **serum at midcycle**
  - in HP hMG cycles higher levels of
    - estradiol
  - in rFSH cycles higher levels of
    - progesterone

- **follicular fluid**
  - in HP hMG cycles higher levels of
    - LH, FSH, hCG, androstenedione, testosterone
    - $E_2:A$, $E_2:T$, $E_2:P$ ratios
  - in rFSH cycles higher levels of
    - progesterone
Recent clinical comparisons between FSH and hMG/HP hMG

  - metanalysis
  - higher clinical pregnancy rates in hMG vs. FSH treatment
  - no difference in ongoing pregnancy or live birth rates

- **Platteau et al**, *Fertil Steril* 81:1401, 2004
  - better pregnancy outcome in IVF patients treated with HP hMG vs. rFSHα
  - no difference in ICSI patients

  - trend (statistically nonsignificant) towards higher pregnancy rates in IVF patients treated with HP hMG (27%) vs. rFSHα (22%)
A novel chimeric recombinant gonadotropin (C3) with both FSH and hCG activity

SDS-PAGE and RP-HPLC of C3 and rhCG

Garone et al
Endocrinology
147:4205, 2006
Rationale for LH activity supplementation

- Estrogen effects on oocyte
- Modulation of folliculogenesis
- FSH synergies
- FSH replacement
Proposed ovarian stimulation regimen
Filicori and Cognigni, JCE&M, 86:1437, 2001

Exogenous gonadotropin dose

- Early FSH/hMG
- Mid low-dose hCG/rLH
- Late high-dose hCG

Follicular phase
Protocol scheme
Filicori et al, Fertil Steril 84:394, 2005

- Depot triptorelin 3.75 mg
- rFSH/hMG
- hCG 10,000 IU
- > 6 follicles > 12mm and E2 > 600 pg/mL

Group A (24 pts)
- rFSH/hMG
- OPU
- ICSI

Group B (24 pts)
- rFSH/hMG
- hCG 200 IU/day
- OPU
- ICSI

depot triptorelin 3.75 mg
Filicori et al, Fertil Steril 84:394, 2005
Preovulatory follicles
(before hCG 10,000 IU)

Felicori et al
Fertil Steril
84:394, 2005
Follicular fluid estrogen/androgen ratios

Filicori et al
Fertil Steril
84:394, 2005
## Clinical outcome
*Filicori et al, Fertil Steril 84:394, 2005*

<table>
<thead>
<tr>
<th></th>
<th>group A (no hCG)</th>
<th>group B (hCG)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>overall treatment duration</td>
<td>days</td>
<td>11.6±0.2</td>
<td>11.9±0.1</td>
</tr>
<tr>
<td>rFSH/hMG duration</td>
<td>days</td>
<td>11.6±0.2</td>
<td>8.6±0.1</td>
</tr>
<tr>
<td>daily hCG duration</td>
<td>days</td>
<td>-</td>
<td>3.3±0.1</td>
</tr>
<tr>
<td>rFSH/hMG dose</td>
<td>IU</td>
<td>2,779±160</td>
<td>1,960±99</td>
</tr>
<tr>
<td>gonadotropin cost</td>
<td>€</td>
<td>1,146±66</td>
<td>808±41</td>
</tr>
<tr>
<td>mature oocytes</td>
<td>n</td>
<td>8.0±0.8</td>
<td>8.2±0.6</td>
</tr>
<tr>
<td>fertilization rates</td>
<td>%</td>
<td>48±4</td>
<td>74±3</td>
</tr>
<tr>
<td>good quality embryos</td>
<td>%</td>
<td>86±6</td>
<td>84±5</td>
</tr>
<tr>
<td>embryos transferred</td>
<td>n</td>
<td>2.3±0.2</td>
<td>2.5±0.1</td>
</tr>
<tr>
<td>pregnancy rates</td>
<td>%</td>
<td>21</td>
<td>25</td>
</tr>
</tbody>
</table>
## Selective use of LH activity in the late stages of ovulation induction and COS

<table>
<thead>
<tr>
<th>Study</th>
<th>Type/Dose</th>
<th>Regimen</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filicori, 2002</td>
<td>hCG 200 IU</td>
<td>COS</td>
<td>LFP low dose hCG alone supports folliculogenesis and steroidogenesis</td>
</tr>
<tr>
<td>Filicori, 2002</td>
<td>hCG 200 IU</td>
<td>COS</td>
<td>first pregnancy with low dose hCG alone in LFP</td>
</tr>
<tr>
<td>Fabregues, 2003</td>
<td>rLH 375 IU</td>
<td>OI</td>
<td>pregnancy with rLH alone in LFP in HH pt</td>
</tr>
<tr>
<td>Lee, 2005</td>
<td>hCG 200 IU</td>
<td>PCOS</td>
<td>prevention of OHSS</td>
</tr>
<tr>
<td>Branigan, 2005</td>
<td>hCG 200 IU</td>
<td>Clomid OI</td>
<td>applicable in Clomid OI</td>
</tr>
<tr>
<td>Filicori, 2005</td>
<td>hCG 200 IU</td>
<td>COS</td>
<td>applicability confirmed in clinical setting</td>
</tr>
<tr>
<td>Kenigsberg, 2006</td>
<td>rCG 8 µg</td>
<td>COS-ant</td>
<td>applicability in antagonist cycles</td>
</tr>
<tr>
<td>Serafini, 2006</td>
<td>hCG 200 IU</td>
<td>COS-ant</td>
<td>clinical applicability in antagonist cycles</td>
</tr>
<tr>
<td>Gomes, 2006</td>
<td>hCG 200 IU</td>
<td>COS</td>
<td>reduced cost of COS</td>
</tr>
<tr>
<td>Koichi, 2006</td>
<td>hCG 200 IU</td>
<td>COS-ant</td>
<td>trends toward reduced OHSS</td>
</tr>
</tbody>
</table>
Rationale for LH activity supplementation

Summary

- there is no proof of untoward actions of LH activity in ovarian stimulation
- LH activity supplementation:
  - is critical for ovarian stimulation in hypogonadotropic hypogonadism
  - can benefit GnRH analog-treated patients, but impact of residual endogenous LH secretion is difficult to ascertain
- use of LH activity in ovarian stimulation provides a more balanced follicle response pattern and can limit progesterone secretion
- proper use of LH activity in COS can:
  - lower FSH dose requirements
  - enhance serum and intrafollicular estrogen activity
  - reduce serum and intrafollicular progesterone levels
  - optimize folliculogenesis and reduce small preovulatory follicles
  - allow to limit FSH administration in the last COS stages
Do we need to add LH/hCG for ovarian stimulation?

**Conclusions**

**Do we have proof that:**

- LH activity is a fundamental component of physiologic and stimulated cycles?
  - Yes, abundantly

- We need LH activity in ovarian stimulation?
  - Yes, but source (endogenous or exogenous), type (LH or hCG), and dose required is still controversial

- Novel LH activity administration regimens could drastically optimize ovarian stimulation?
  - Yes, and late follicular phase FSH replacement with LH/hCG appears most promising, though for commercial reasons it may never gain wide acceptance

**So, what about certainty?**

- Leave it to the creationists!