LH ad back in ART – do we need it

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Overview

- ICOS definition

- Molecular and functional differences between LH and hCG

- Studies showing an effect of LH supplementation in subgroups

- Hypotheses as to the effect of LH supplementation in subgroups

- The issue of late follicular phase progesterone rise
Serum LH in GnRHa protocol versus the natural cycle

Westergaard et al., 1998
iCOS concept:

There is no "standard patient" in ART
Treatment tailored to the needs of the patient

- GnRH analogue, FSH dose/duration, +/- LH activity
- Ovulation trigger - HCG or GnRHa
- Embryo selection - subjective → objective criteria
- Luteal phase support
So what about LH activity supplementation?

- LH supplementation is mandatory in the hypogonadotropic hypogonadal (HH) patient (LH < 1.2 IU/l)

- For most women the endogenous LH level after down-regulation is sufficient for follicular development and steroidogenic activity

- FSH-only - well established successful protocol in ART
LH activity - LH and/or hCG in LH containing gonadotropins

- 75 IU rLH:
  75 IU LH

- 75 IU HMG:
  75 IU FSH + 75 IU LH “activity”
  (10-12 IU hCG + 4 IU natural LH)

Does it make a difference?
Peptide composition of gonadotropins

<table>
<thead>
<tr>
<th></th>
<th>α</th>
<th>β</th>
<th>M&lt;sub&gt;w&lt;/sub&gt;</th>
<th>No. of Amino-acids</th>
<th>Sequence homology with LH</th>
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<tbody>
<tr>
<td>LH</td>
<td></td>
<td>α</td>
<td>28.000</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td>hCG</td>
<td>α</td>
<td>β</td>
<td>37.000</td>
<td>145</td>
<td>81%</td>
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<tr>
<td>FSH</td>
<td></td>
<td>β</td>
<td>28.000</td>
<td>117</td>
<td>41%</td>
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## Characteristics of gonadotropins

<table>
<thead>
<tr>
<th></th>
<th>FSH</th>
<th>LH</th>
<th>hCG</th>
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</thead>
<tbody>
<tr>
<td>No. of sugar residues</td>
<td>4</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Terminal half life</td>
<td>24 hours</td>
<td>21-24 hours</td>
<td>72-96 hours</td>
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<tr>
<td>Chromosome localization of the gene for the β-chain</td>
<td>11</td>
<td>19q13.3</td>
<td>19q13.3</td>
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<tr>
<td>No. of copies of the gene</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>
LH and hCG structural differences

Anterior Pituitary Gland

LH

Trophoblastic embryonic cells

hCG
Are LH and hCG equivalent - gene expression?

LHR and FSHR expression
(Trafficking of retinoic acid : RXRB, TTR, ALDH8A1)

Meiosis and follicular maturation
(TRA : RXRB, TTR, ALDH8A1; IL11; AKT3)

Follicular development (IL11; AKT3)

Cellular growth (RXRB, TTR, ALDH8A1; IL11; AKT3)

Ovarian stereodogenesis
(TRA : RXRB, TTR, ALDH8A1)

Embryo development & survival (AKT3)

Inibition of aromatase
(PPARS)

Apoptosis enhancement
(DDNAsi)
LH versus hCG activity

Although similar in action - significant differences exist between LH and hCG at the:

- **Structural level**
- **Molecular level**
- **Functional level**
Does it show whether hCG (HMG) or FSH?  
Gene expression

- 30 IVF/ICSI patients randomized to rFSH or HMG treatment
- At aspiration granulosa cells collected for gene expression analysis

Results:
- 85 genes statistically significantly different in expression

Grønlund ML et al., Fert Ster 2008
Does it show whether hCG (HMG) or FSH?

Results:
- Expression levels of LH/hCG receptor gene and genes involved in biosynthesis of cholesterol and steroids were expressed at a lower level in HMG-treated granulosa cells.

Conclusion:
- Preparation used for COS may impact the developmental competence of the oocyte and the function of the corpus luteum.

Grønlund ML et al., Fert Ster 2008
Meta-analyses on HMG versus rFSH

- Meta-analyses on r-hFSH versus hMG:
  - Daya S, 2002: better pregnancy rate with r-hFSH
  - Van Wely et al., 2003: better pregnancy rates with hMG
  - Al-Inany at al., 2003; 2005: no difference in pregnancy/live birth rate
  - Coomrasay, 2008: better live birth rate with hMG
Meta-analysis

Why these confusing differences?

- Differences in strictness of inclusion criteria, methodology and design
- Inclusion criteria of papers designed to arrive at a desired conclusion
- Conclusions of a meta-analysis - no better than the studies included
Recombinant human follicle-stimulating hormone produces more oocytes with a lower total dose per cycle in assisted reproductive technologies compared with highly purified human menopausal gonadotrophin: a meta-analysis

Philippe Lehert¹, Joan C Schertz², Diego Ezcurra³*
Meta-analysis 2010

- Large meta-analysis comparing r-hFSH and hMG 4040 cycles from 16 studies out of 30 evaluated

- **Selection:**
  All published randomized controlled trials on ovarian stimulation comparing the two gonadotropin products evaluated

Lehert et al., 2010
When comparing rFSH vs HMG:

- Same pregnancy rate in fresh transfers
- More oocytes produced with r-hFSH compared with hMG
- Less gonadotropins utilized with r-hFSH (0.7 > oocytes /1000 IU)
- Drug efficiency should be evaluated per cycle of stimulation including pregnancies achieved with fresh + frozen/thawed embryos (cumulative PR)

Lehert et al., 2010
Cochrane Meta-analysis 2012
Oocytes, rFSH versus FSH and LH activity

Figure 5

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Mean (SD) FSH only</th>
<th>Mean (SD) FSH + LH activity</th>
<th>Mean Difference IV, Random, 95% CI</th>
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</thead>
<tbody>
<tr>
<td>Andersen 2006</td>
<td>11.8 (5.7)</td>
<td>10 (5.4)</td>
<td>1.80 [1.00, 2.60]</td>
</tr>
<tr>
<td>Balasch 2001</td>
<td>10.1 (1.1)</td>
<td>8.4 (0.9)</td>
<td>1.70 [0.95, 2.45]</td>
</tr>
<tr>
<td>Balasch 2003</td>
<td>11.79 (4.55)</td>
<td>9.1 (4.35)</td>
<td>2.69 [0.22, 5.16]</td>
</tr>
<tr>
<td>Barrenetxea 2006</td>
<td>5.66 (0.64)</td>
<td>5.42 (0.55)</td>
<td>0.24 [-0.04, 0.52]</td>
</tr>
<tr>
<td>Bosch 2008</td>
<td>14.4 (8.1)</td>
<td>11.3 (6)</td>
<td>3.10 [1.33, 4.87]</td>
</tr>
<tr>
<td>Esteves 2007</td>
<td>10.75 (6.04)</td>
<td>11.21 (5.91)</td>
<td>-0.46 [-1.65, 0.73]</td>
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<tr>
<td>Grondahl 2009</td>
<td>13.2 (1.6)</td>
<td>9.8 (1.6)</td>
<td>3.40 [2.25, 4.55]</td>
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<tr>
<td>Hompes 2008</td>
<td>10.56 (7.8)</td>
<td>7.76 (5.92)</td>
<td>2.80 [1.54, 4.06]</td>
</tr>
<tr>
<td>Kilani 2003</td>
<td>6.8 (4.24)</td>
<td>7.9 (4.95)</td>
<td>-1.10 [-2.91, 0.71]</td>
</tr>
<tr>
<td>Matorras 2009</td>
<td>8.9 (4.9)</td>
<td>8.3 (4.7)</td>
<td>0.60 [-1.04, 2.24]</td>
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<tr>
<td>Serhal 2000</td>
<td>8.3 (4.3)</td>
<td>9.5 (4.4)</td>
<td>-1.20 [-2.33, -0.07]</td>
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<td>Strehler 2001</td>
<td>12.29 (7.8)</td>
<td>9.67 (5.92)</td>
<td>2.62 [1.42, 3.82]</td>
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<tr>
<td>Strowitzki 2007</td>
<td>7.1 (3.9)</td>
<td>5.4 (4.9)</td>
<td>1.70 [-0.54, 3.94]</td>
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<tr>
<td>Tarlatzis 2006</td>
<td>9.8 (7)</td>
<td>10.1 (5.4)</td>
<td>-0.30 [-2.61, 2.01]</td>
</tr>
</tbody>
</table>

Total (95% CI) 1572 1581 100.0% 1.25 [0.48, 2.02]

Heterogeneity: Tau² = 1.64; Chi² = 95.88, df = 13 (P < 0.00001); I² = 86%
Test for overall effect: Z = 3.17 (P = 0.002)

Number of oocytes retrieved.

Al Inany et al., 2012
LH supplementation in ART

- Controverted topic
- Confusing evidences
- Lack of consensus
  - No benefit in unselected population
  - Potential benefit in (initial) poor response
  - Profound LH suppression in GnRH agonist long protocol
  - Better outcome in patients > 35 years old

Mochtar et al, 2007 Cochrane Database Syst Rev.18: 2
Use of LH Supplementation in ART

Beneficial effect of LH supplementation in sub-groups

- **Age**
  - Bosch et al. 2011, Matorras et al., 2009
  - Marrs et al., 2004 Humaidan et al., 2004

- **Initial poor responders**
  - Barrenatexea 2000
  - Placido et al., 2004

- **Follicular stagnation**
  - Ferraretti et al., 2004

- **Initial poor responders**
  - Ruvolo et al., 2007
## Comparative studies rFSH vs rFSH + rLH according to age

<table>
<thead>
<tr>
<th>GnRH Agonist</th>
<th>&lt; 35 years old</th>
<th>≥ 35 years old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marrs et al, 2004</td>
<td>FSH = FSH + LH (n=310)</td>
<td>FSH + LH &gt; FSH (n=88)</td>
</tr>
<tr>
<td>Humaidan et al, 2004</td>
<td>FSH = FSH + LH (n=192)</td>
<td>FSH + LH &gt; FSH (n=38)</td>
</tr>
<tr>
<td>NyboeAndersen et al, 2008</td>
<td>FSH = FSH + LH (n=426)</td>
<td>FSH + LH = FSH (n=100)</td>
</tr>
<tr>
<td>Fábregues et al, 2006</td>
<td></td>
<td>FSH + LH &gt; FSH (n=120)</td>
</tr>
<tr>
<td>Matorras et al, 2009</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>GnRH Antagonist</th>
<th>&lt; 35 years old</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Sauer et al , 2004</td>
<td>FSH = FSH + LH (n=49)</td>
<td>FSH + LH &gt; FSH (n=292)</td>
</tr>
<tr>
<td>Griesinger et al, 2005</td>
<td>FSH = FSH + LH (n=126)</td>
<td></td>
</tr>
<tr>
<td>Levi-Setti et al, 2006</td>
<td>FSH = FSH + LH (n=40)</td>
<td></td>
</tr>
<tr>
<td>Bosch et al., 2011</td>
<td>FSH = FSH + LH (n=333)</td>
<td></td>
</tr>
</tbody>
</table>

Increased IR in women > 35 years of age
43 studies

6443 patients (r-hFSH plus r-hLH, n = 3113; r-hFSH, n = 3228)

Conclusion:
Significantly higher clinical pregnancy rates were observed with r-hFSH plus r-hLH versus r-hFSH alone in the overall population analysed in this review (risk ratio [RR] 1.09; 95% CI 1.01-1.18) and in poor responders (n = 1179; RR 1.30; 95% CI 1.01-1.67; intention-to-treat population)

Lehert et al., 2014
LH Supplementation in ART

- Ovarian ageing - hypotheses as to the effect of LH supplementation?

- A question of androgens and the anti-apoptotic effect of LH?
The ageing ovary - endocrinological changes

n = 1423

- Total Testosterone ↓ 55%
- DHEAS ↓ 77%
- Free Testosterone ↓ 49%
- Androstenedione ↓ 64%

Davison SL et al JCEM 2005;90:3847
Primate folliculogenesis

Its ”all about androgens”

- FSH receptor induction in granulosa cells – responsiveness ↑
  (Weil et al., 1999)

- Act synergistically with IGF1– growth ↑
  (Vendola et al., 1999)

- Increase in pre-antral and antral follicles – recruitability ↑
  (Vendola et al., 1998; 1999; Spinder et al., 1989)
Differential effects of LH activity according to the stage of folliculogenesis

Theca cell

- LH
- C → 17-OH P

Granulosa cell

- FSH, LH
- A4 → E2
- Aromatization

Early follicular phase

- LH supplementation
  - increases androgen synthesis
  - stimulates follicular recruitment

Mid-follicular phase (follicle > 8-12 mm)

- LH supplementation
  - increases oestrogen synthesis
  - stimulates follicular growth
Ovarian ageing and cumulus cell apoptosis

- Cumulus cells surround and intercommunicate with the oocyte during follicular development

- High levels of apoptotic granulosa cells associated with low quality embryos (Høst et al., 2000; Lee et al., 2001)

- Apoptosis rate in cumulus cells significantly increased with increasing age (Lee et al., 2001; Bencomo et al., 2006)
Growth factors and LH supplementation

FGF2 - one of the most prominent factors for angiogenesis, located in theca and granulosa cell

Growth factors: amphiregulin (AR) and epiregulin (Ep) present in granulosa cells

- Upregulated by LH
  
  (Rimon E et al., 2004; Robinson RS et al., 2007)

- Anti-apoptotic effect on granulosa cells
  
  (Tilly JL et al., 1992; Peluso JJ et al., 2001, Ben-Ami I et al., 2009)
LH Supplementation and apoptosis in cumulus cells

Ruvulo et al. (2007) - apoptosis rate in cumulus cells

"Initial poor responders" in a previous FSH only cycle

42 patients– randomised into 2 arms:

From cd 8  FSH +150 IU LH - or FSH only

- Apoptosis in cumulus cells ↓
- Immature oocytes ↓
- Transferable embryos ↑
- PR and IR ↑
Initial poor responder patients

Cochrane review 2007
r-hFSH alone vs r-hLH + r-hFSH

Favours r-hFSH

Favours r-hFSH + r-hLH
Use of LH Supplementation in ART

Patients with a suboptimal response to FSH - 12-14 % of patients
(Barrenatexea et al., 2000; Placido et al., 2004; Ferraretti et al., 2004; Ruvolo et al., 2007)
LH Supplementation in ART

- Suboptimal response to FSH only - hypotheses as to the effect of LH supplementation?
- FSH and LH work in synergy

Reduced bioactivity of endogenous LH?
LH Supplementation in ART

- **Polymorphism:**
  Gene DNA variant existing in the normal population at a frequency of 1% or more

- **Mutation:**
  Gene DNA variant existing in the normal population at a frequency of less than 1%
LH Supplementation in ART

V-LHβ - LH gene polymorphism

- Carrier frequency 0-52 % in various ethnic groups
- Frequency 13 % in Denmark
- Frequency 12-13 % in Italy

Reduced bioactivity

(Alviggi and Humaidan, 2013; Huhtaniemi et al., 1999; Jiang et al., 1999; Ropelato et al., 1999)
V-LH polymorphism in women with resistance to FSH: An observational retrospective study
Alviggi C (Italy), Petterson K (Finland), and Humaidan P (Denmark)

60 patients screened for V-LHβ:

- **Group A**: 22 patients > 3500 IU rFSH
- **Group B**: 15 patients 2000-3500 IU rFSH
- **Group C**: 23 patients < 2000 IU rFSH

Alviggi et al RBM Online 2009
LH gene polymorphism in women with ovarian resistance to FSH

- Overall incidence (8/60 – 13.3%)

Group A: 7 carriers of v-LH - 2 homozygotes / 5 heterozygotes (31.8%)

Group B: 1 carrier of v-LH – heterozygote (6.6%)

Group C: No carrier
LH Supplementation in ART

Ovarian sensitivity to FSH is a polygenic trait
Future scenario:

Pharmacogenetics

Compiling data in one chip to phenotype patients prior to COS:

V-LHβ (LH gene polymorphism; 12-50%) (Lamminen et al., 2001)
FSH-R gene polymorphism (14%) (Mayorga et al., 2000)
LH-R gene polymorphism (?)
AMH and AMH-R gene polymorphism (Kevenaar et al., 2007)
ESR1 gene polymorphism (Altmae et al., 2007; Georgiou et al., 1997)
LH activity supplementation in 2014

- LH activity supplementation only for two sub-groups of normogonadotrophic patients

- Patients > 35 years of age
  (Marrs et al., 2004; Humaidan et al., 2004; Matorras et al., 2009; Bosch et al. 2011)

- Patients with a suboptimal response to ”FSH only” 12-14% of patients
  (Barrenatexea et al., 2000; de Placido et al., 2004; Ferraretti et al., 2004; Ruvolo et al., 2007)

- Optimal starting day – day 1 of stimulation
Overview

- ICOS definition

- Molecular and functional differences between LH and hCG

- Studies showing an effect of LH supplementation in subgroups

- Hypotheses as to the effect of LH supplementation in subgroups

- The issue of late follicular phase progesterone rise
Natural menstrual cycle
How is follicular progesterone production regulated during controlled ovarian stimulation?

C Yding Andersen
• A late follicular phase progesterone level above 1.5 ng/ml compromises the pregnancy rate in all COS cycles

• In all cycles with late follicular phase progesterone levels above 1.5 ng/ml a freeze all policy should be adopted

Venetis et al., 2013
Bosch et al., 2010
Papanikolaou et al., 2009
Nyboe Andersen et al., 2006
• The majority of P4 in circulation (95%) is produced in the intra-follicular compartment by theca and granulosa cells

• Intra-follicular P4 and hydroxy-P4 are terminal products which are not converted into androgens by theca cells and subsequently into estradiol by granulosa cells under the effect of LH/hCG

• The main driver of the production of P4 in the follicular compartment is an increase in FSH and LH or hCG

• Late follicular phase P4 rise is related to number of follicles developed and oocytes retrieved and the effect on the reproductive outcome is still controversial....

Yding Andersen et al., RBM Online 2011
Incidence 4.5% in low responder – 19.0% in high responder

Fertil Steril 2013
**Progestosterone elevation and probability of pregnancy after IVF: a systematic review and meta-analysis of over 60 000 cycles**

C.A. Venetis*, E.M. Kolibianakis, J.K. Bosdou, and B.C. Tarlatzis

- 17% of cycles had late follicular phase P4 rise (> 1.5 ng/ml)
- Less frequent in GnRH antagonist cycles
- ↑ oocytes, ↑ FSH consumption, ↑ E2 → P4 ↑
- LH activity does not reduce P4 rise!
Clinical implications of Meta-analyses (60,000 cycles)

In an IVF unit with 1000 cycles yearly:

- Monitor 1000 cycles for progesterone
- Intervene in 172 cycles
- Gain 17 pregnancies

- 1000 cycles/year → total reduction in PR from 40% to 38.5% (1.5%)

Is this relevant for daily clinical practise?
Late follicular phase progesterone rise and its consequences –
A fairy tale

Let's move on...
Thank You for Your attention
peter.humaidan@midt.rm.dk