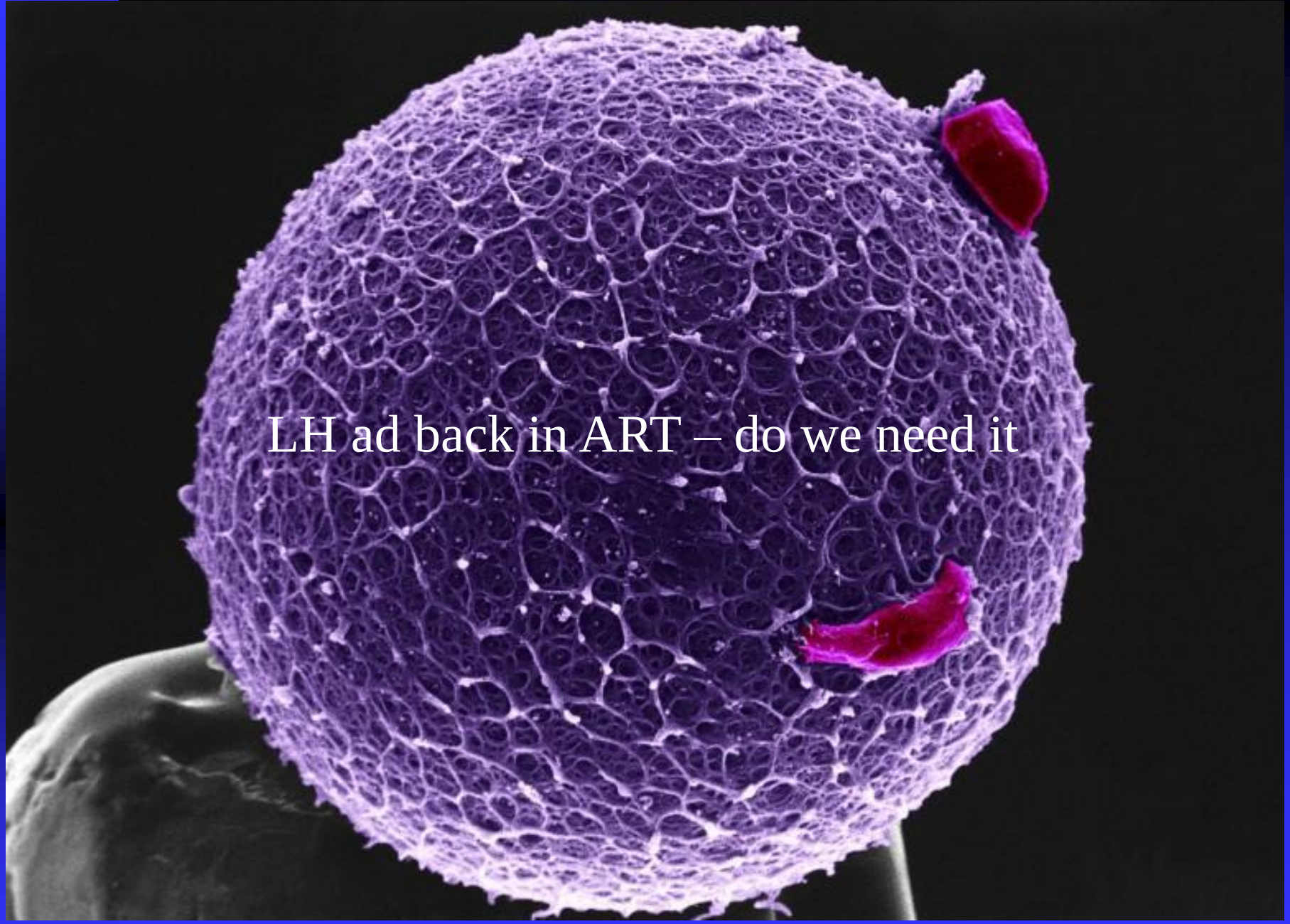
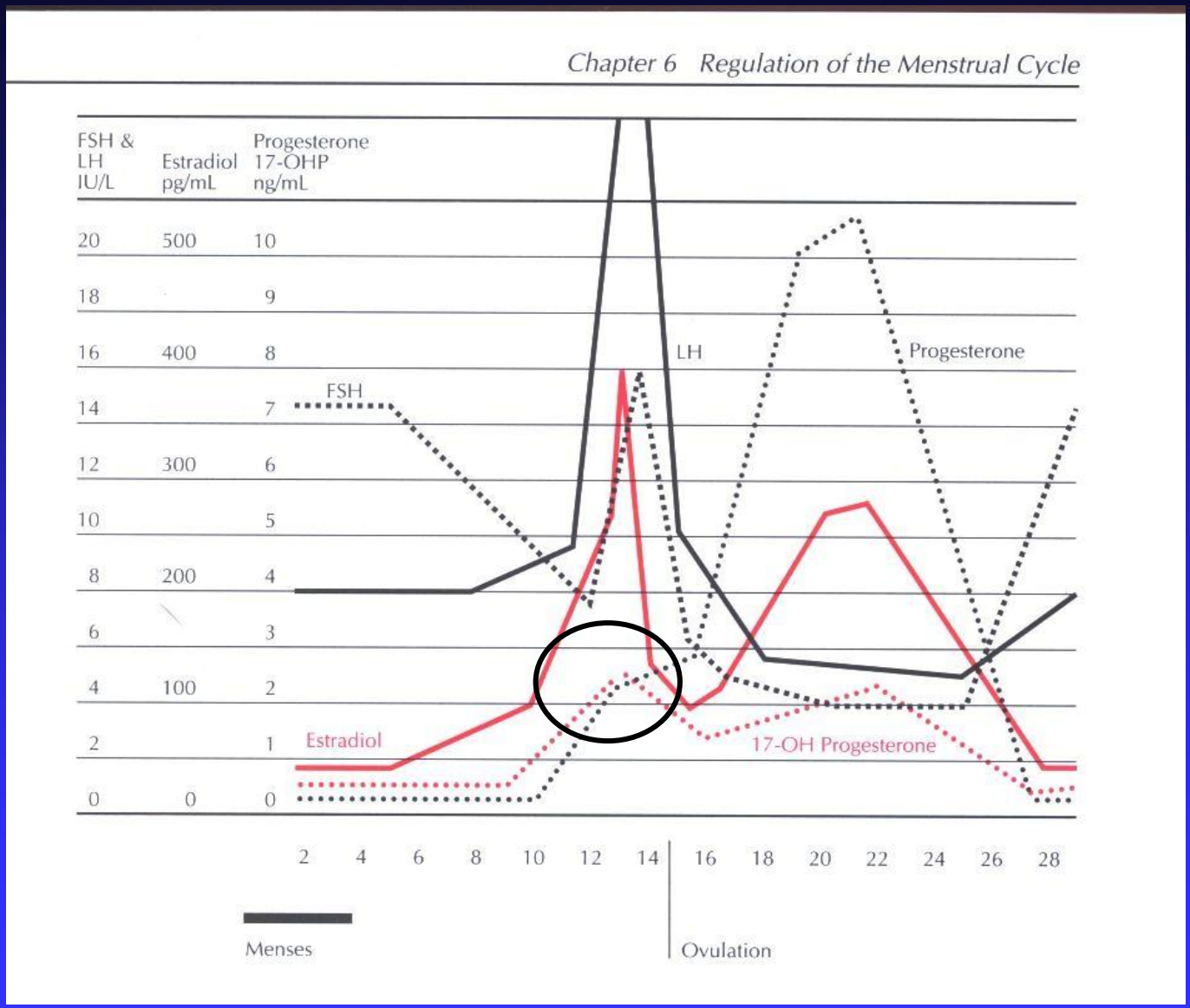



Peter Humaidan
Skive Fertility Clinic and Faculty of Health  Aarhus University Denmark

# Overview

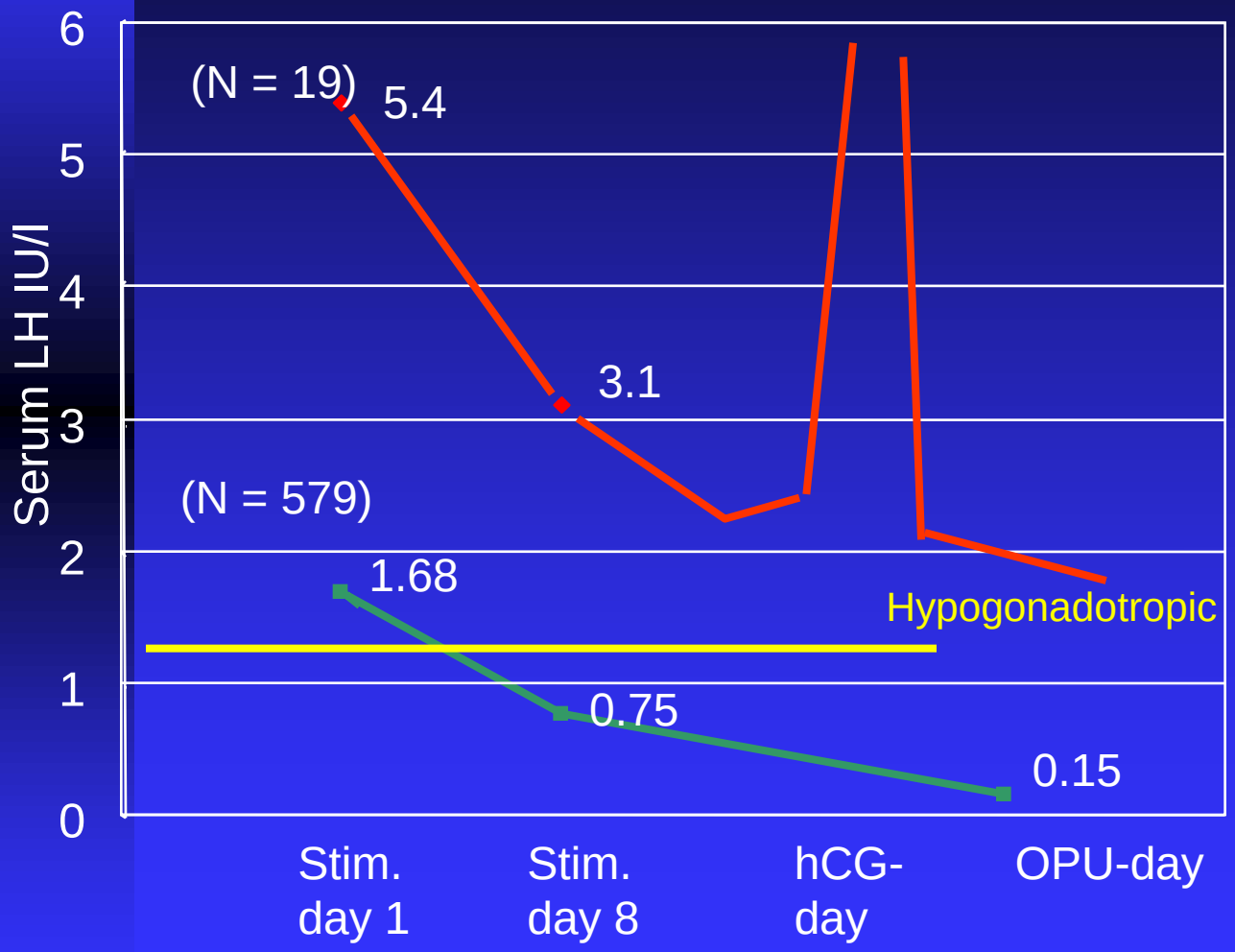
- ICOS definition
- Molecular and functional differences between LH and hCG
- Studies showing an effect of LH supplementation in subgroups
- Hypotheses as to the effect of LH supplementation in subgroups
- The issue of late follicular phase progesterone rise

# Natural menstrual cycle




Speroff L et al. 5th Edition

# 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 actvity 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

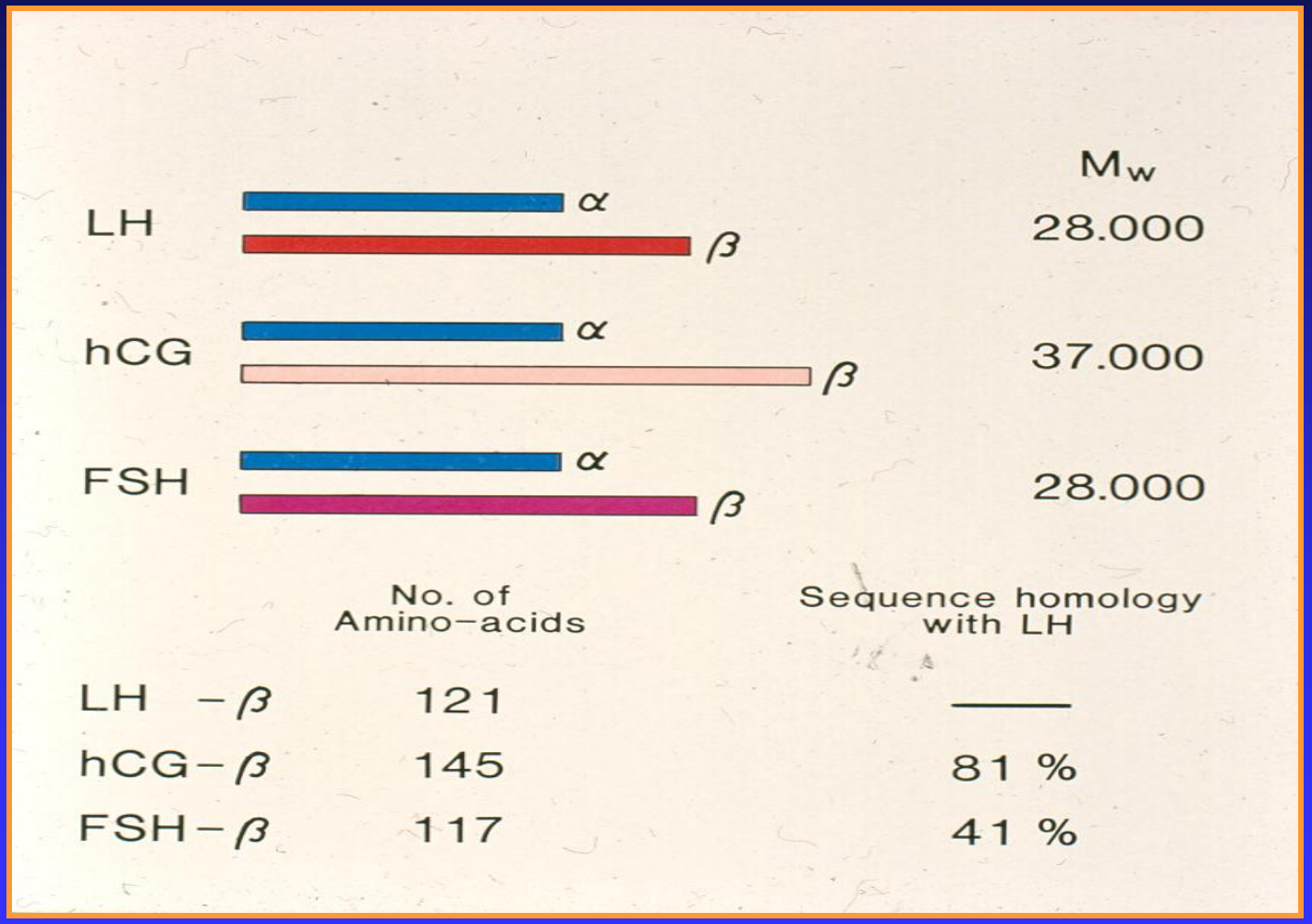
# LH activity - LH and/or hCG in LH containing gonadotropins

- 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

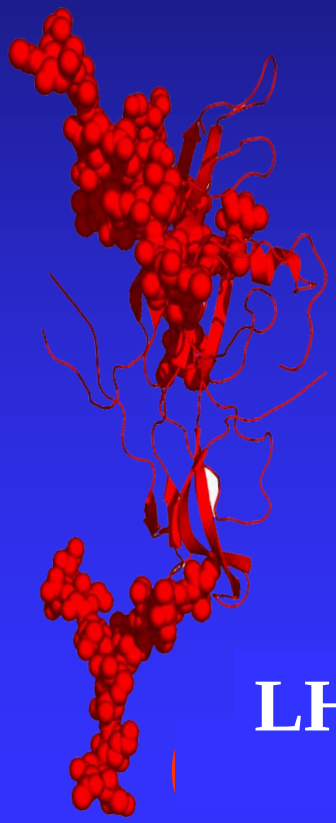


# Characteristics of gonadotropins

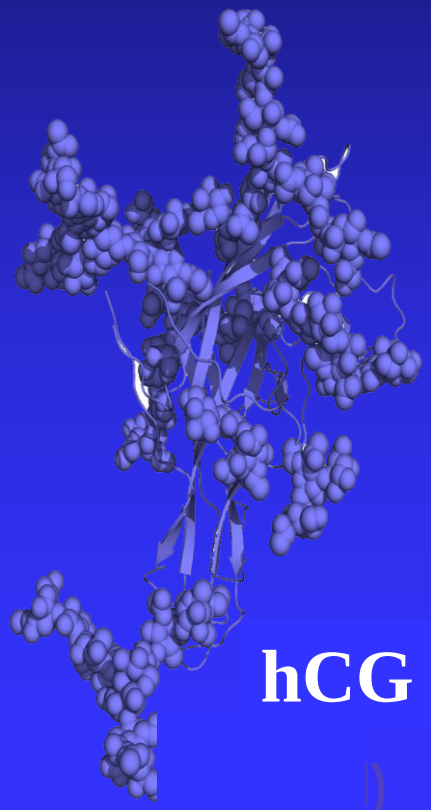
| | FSH | LH | hCG |
|---|---|---|---|
| **No. of sugar residues** | 4 | 3 | 7 |
| **Terminal half life** | 24 hours | 21-24 hours | 72-96 hours |
| **Chromosome localization of the gene for the α-chain** | 6q21.1-23. | 6q21.1-23. | 6q21.1-23. |
| **Chromosome localization of the gene for the β-chain** | 11 | 19q13.3 | 19q13.3 |
| **No. of copies of the gene** | 1 | 1 | 6 |

# LH and hCG structural differences

- Anterior Pituitary Gland
- Trophoblastic embryonic cells



LH



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**
**(DNAsi)**

Società Italiana di Embriologia Riproduzione e Ricerca, data on file 2011

# 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

# Meta-analysis 2010

Lehert *et al. Reproductive Biology and Endocrinology* 2010, **8**:112
http://www.rbej.com/content/8/1/112


**REVIEW** **Open Access**

## 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 Lehert[1], Joan C Schertz[2], Diego Ezcurra[3*]

# 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

# Conclusion of meta-analysis

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

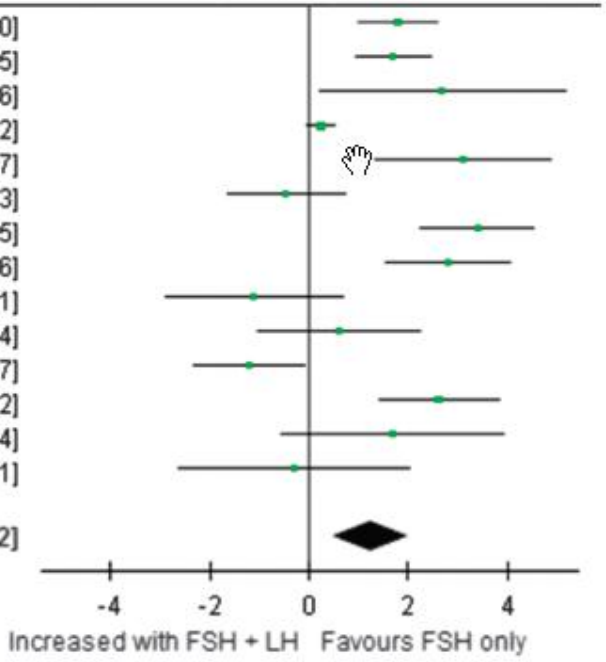
# Cochrane Meta-analysis 2012
# Oocytes, rFSH versus FSH and LH activity

**Figure 5**

| Study or Subgroup | FSH only Mean | SD | Total | FSH + LH activity Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| Andersen 2006 | 11.8 | 5.7 | 368 | 10 | 5.4 | 363 | 8.6% | 1.80 [1.00, 2.60] |
| Balasch 2001 | 10.1 | 1.1 | 13 | 8.4 | 0.9 | 15 | 8.7% | 1.70 [0.95, 2.45] |
| Balasch 2003 | 11.79 | 4.55 | 25 | 9.1 | 4.35 | 25 | 4.8% | 2.69 [0.22, 5.16] |
| Barrenetxea 2006 | 5.66 | 0.64 | 36 | 5.42 | 0.55 | 36 | 9.4% | 0.24 [-0.04, 0.52] |
| Bosch 2008 | 14.4 | 8.1 | 126 | 11.3 | 6 | 122 | 6.3% | 3.10 [1.33, 4.87] |
| Esteves 2007 | 10.75 | 6.04 | 193 | 11.21 | 5.91 | 193 | 7.7% | -0.46 [-1.65, 0.73] |
| Grondahl 2009 | 13.2 | 1.6 | 15 | 9.8 | 1.6 | 15 | 7.9% | 3.40 [2.25, 4.55] |
| Hompes 2008 | 10.56 | 7.8 | 238 | 7.76 | 5.92 | 222 | 7.6% | 2.80 [1.54, 4.06] |
| Kilani 2003 | 6.8 | 4.24 | 50 | 7.9 | 4.95 | 50 | 6.3% | -1.10 [-2.91, 0.71] |
| Matorras 2009 | 8.9 | 4.9 | 68 | 8.3 | 4.7 | 63 | 6.6% | 0.60 [-1.04, 2.24] |
| Serhal 2000 | 8.3 | 4.3 | 94 | 9.5 | 4.4 | 144 | 7.9% | -1.20 [-2.33, -0.07] |
| Strehler 2001 | 12.29 | 7.8 | 259 | 9.67 | 5.92 | 248 | 7.7% | 2.62 [1.42, 3.82] |
| Strowitzki 2007 | 7.1 | 3.9 | 30 | 5.4 | 4.9 | 30 | 5.3% | 1.70 [-0.54, 3.94] |
| Tarlatzis 2006 | 9.8 | 7 | 57 | 10.1 | 5.4 | 55 | 5.1% | -0.30 [-2.61, 2.01] |
| 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
- Initial poor responders — Placido et al., 2004
- Follicular stagnation — Ferraretti et al., 2004
- Initial poor responders — Ruvolo et al., 2007

# Age

## Comparative studies rFSH vs rFSH + rLH according to age

| | | < 35 years old | ≥ 35 years old |
|---|---|---|---|
| **GnRH agonist** | Marrs et al, 2004<br>Humaidan et al, 2004<br>NyboeAndersen et al, 2008<br>Fábregues et al, 2006<br>Matorras et al, 2009 | FSH = FSH + LH (n=310)<br>FSH = FSH + LH (n=192)<br>FSH = FSH + LH (n=426) | FSH + LH > FSH (n=88)<br>FSH + LH > FSH (n=38)<br>FSH + LH = FSH (n=100)<br>FSH + LH = FSH (n=120)<br>FSH + LH > FSH (n=131) |
| **GnRH antagonist** | Sauer et al , 2004<br>Griesinger et al, 2005<br>Levi-Setti et al, 2006<br>Bosch et al., 2011 | FSH = FSH + LH (n=49)<br>FSH = FSH + LH (n=126)<br>FSH = FSH + LH (n=40)<br>FSH = FSH + LH (n=333) | FSH + LH > FSH (n=292) |

**Increased IR in women > 35 years of age**

Reprod Biol Endocrinol. 2014 Feb 20;12(1):17. [Epub ahead of print]

**Recombinant human follicle-stimulating hormone (r-hFSH) plus recombinant luteinizing hormone versus r-hFSH alone for ovarian stimulation during assisted reproductive technology: systematic review and meta-analysis.**

Lehert P, Kolibianakis EM, Venetis CA, Schertz J, Saunders H, Arriagada P, Copt S, Tarlatzis B.

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)

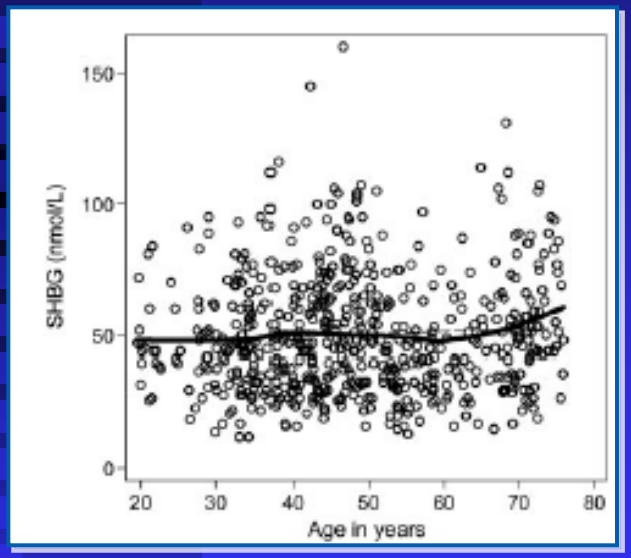
Lehert et al., 2014

# 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

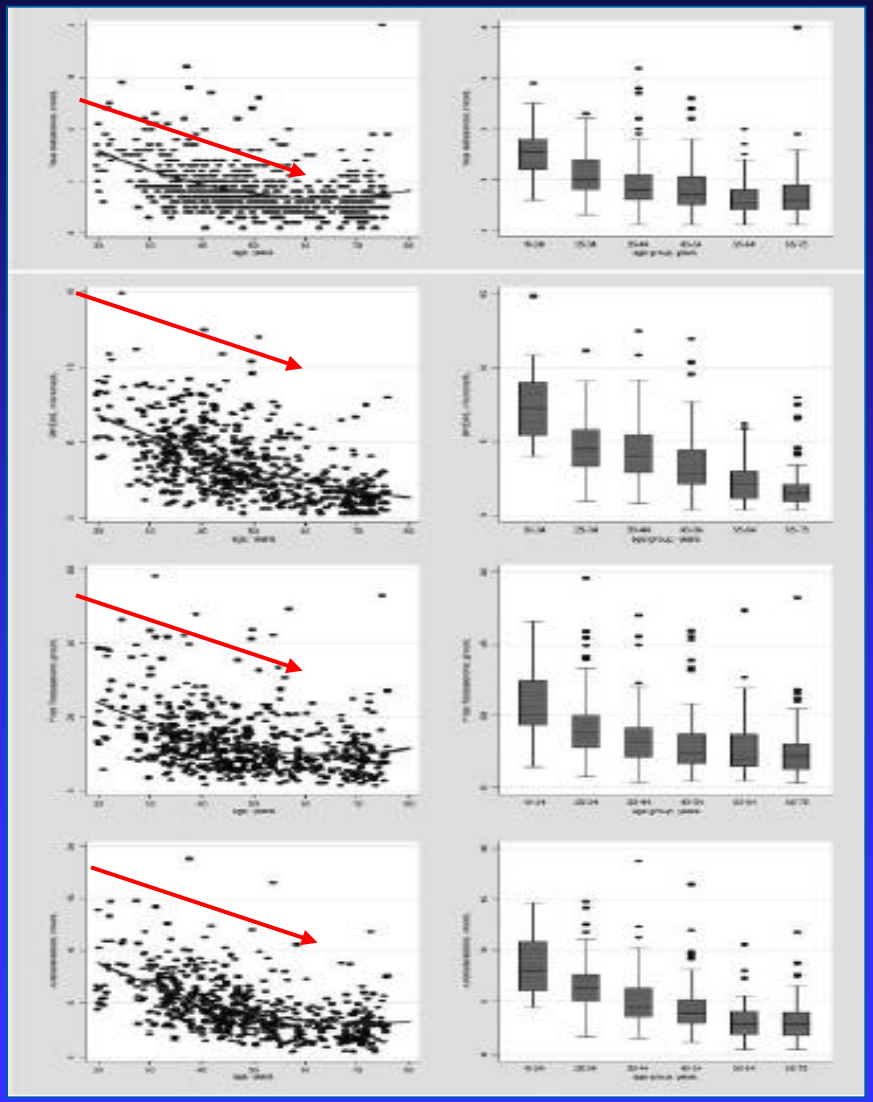



Total Testosterone ↓ 55%

DHEAS ↓ 77%

Free Testosterone ↓ 49%

Androstenedione ↓ 64%



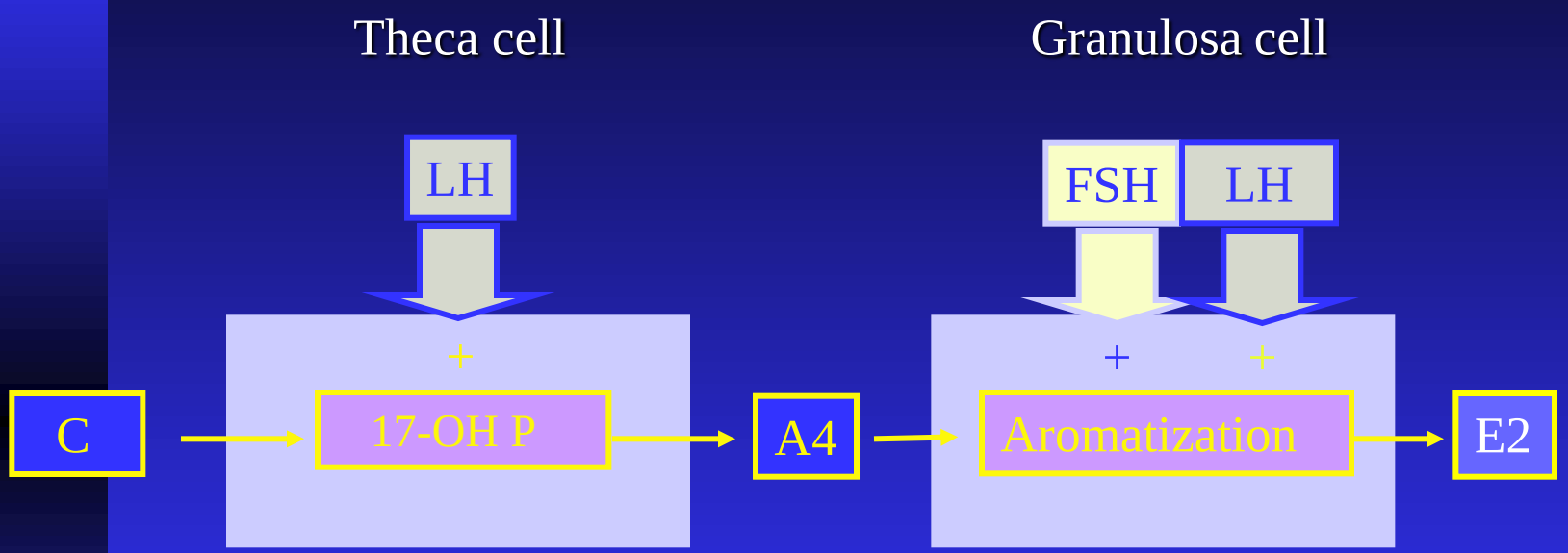
Davison SL et al JCEM 2005;90:3847

# 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




Early follicular phase

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

LH supplementation
- increases androgen synthesis
- stimulates follicular recruitment

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

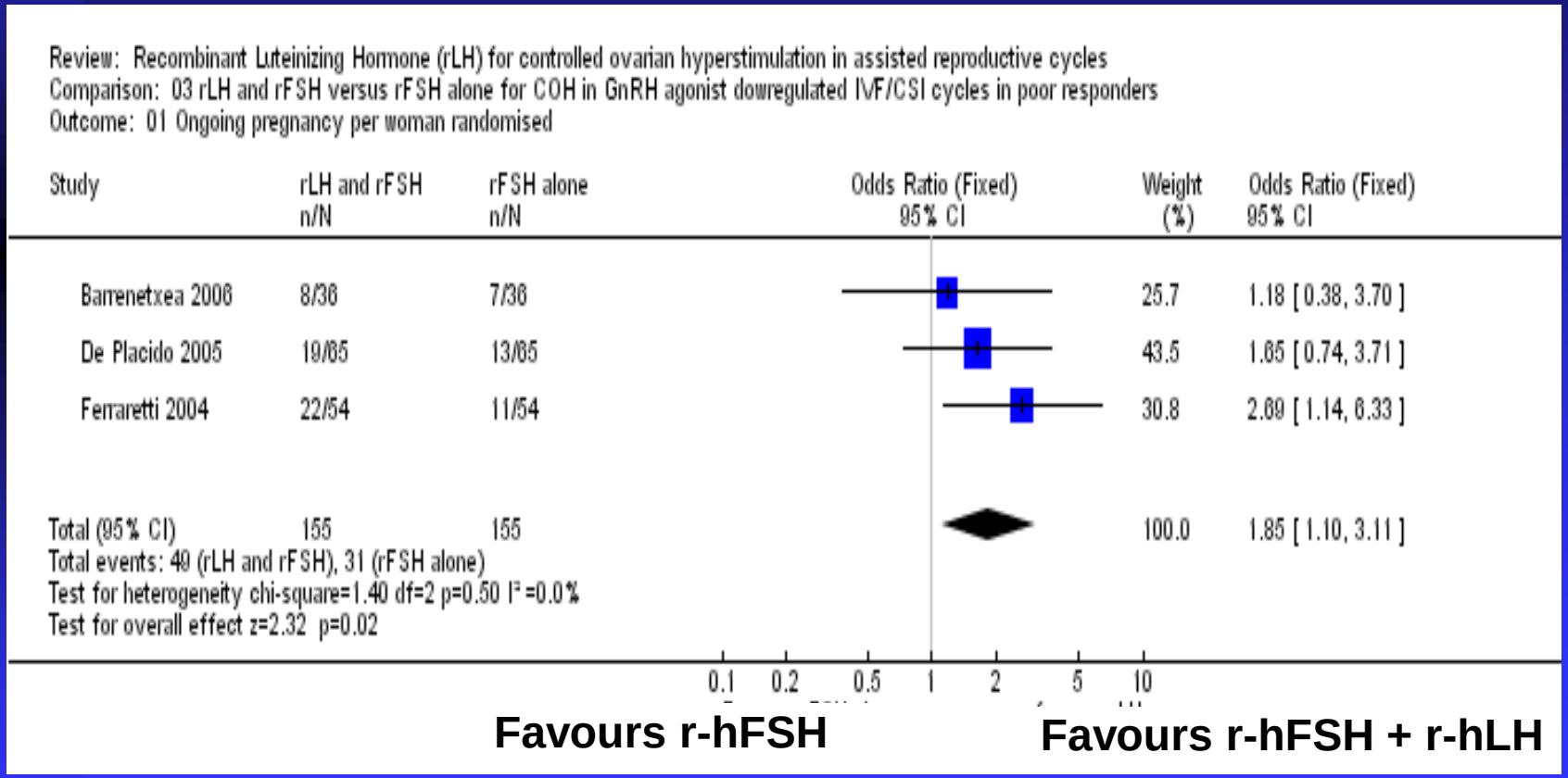
42 patients– randomised into 2 arms:

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



# 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

Alviggi et al RBM Online 2009

# LH Supplementation in ART

Ovarian sensitivity to FSH is a polygenic trait

# LH Supplementation in ART

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 normogonadotropic 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

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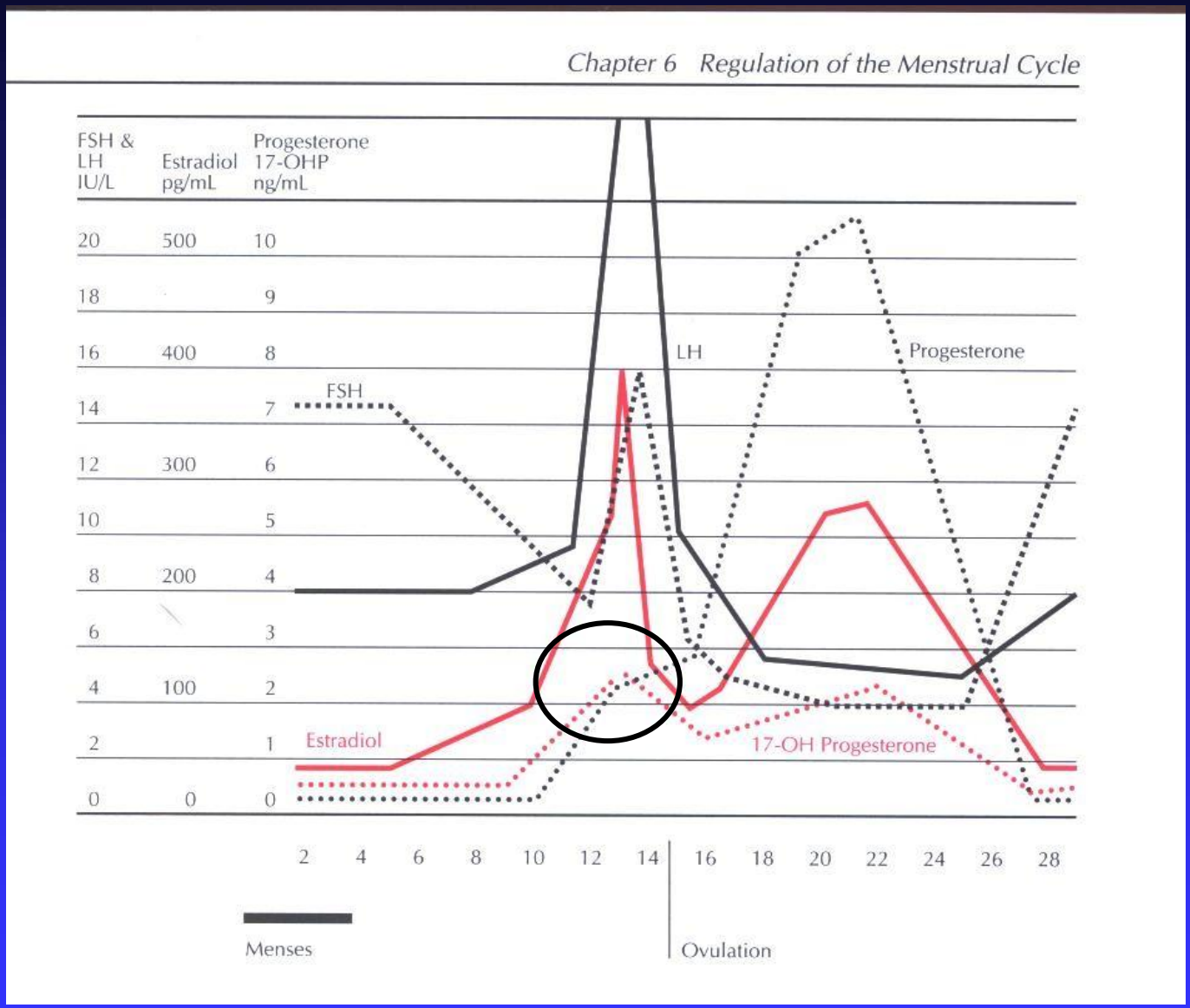
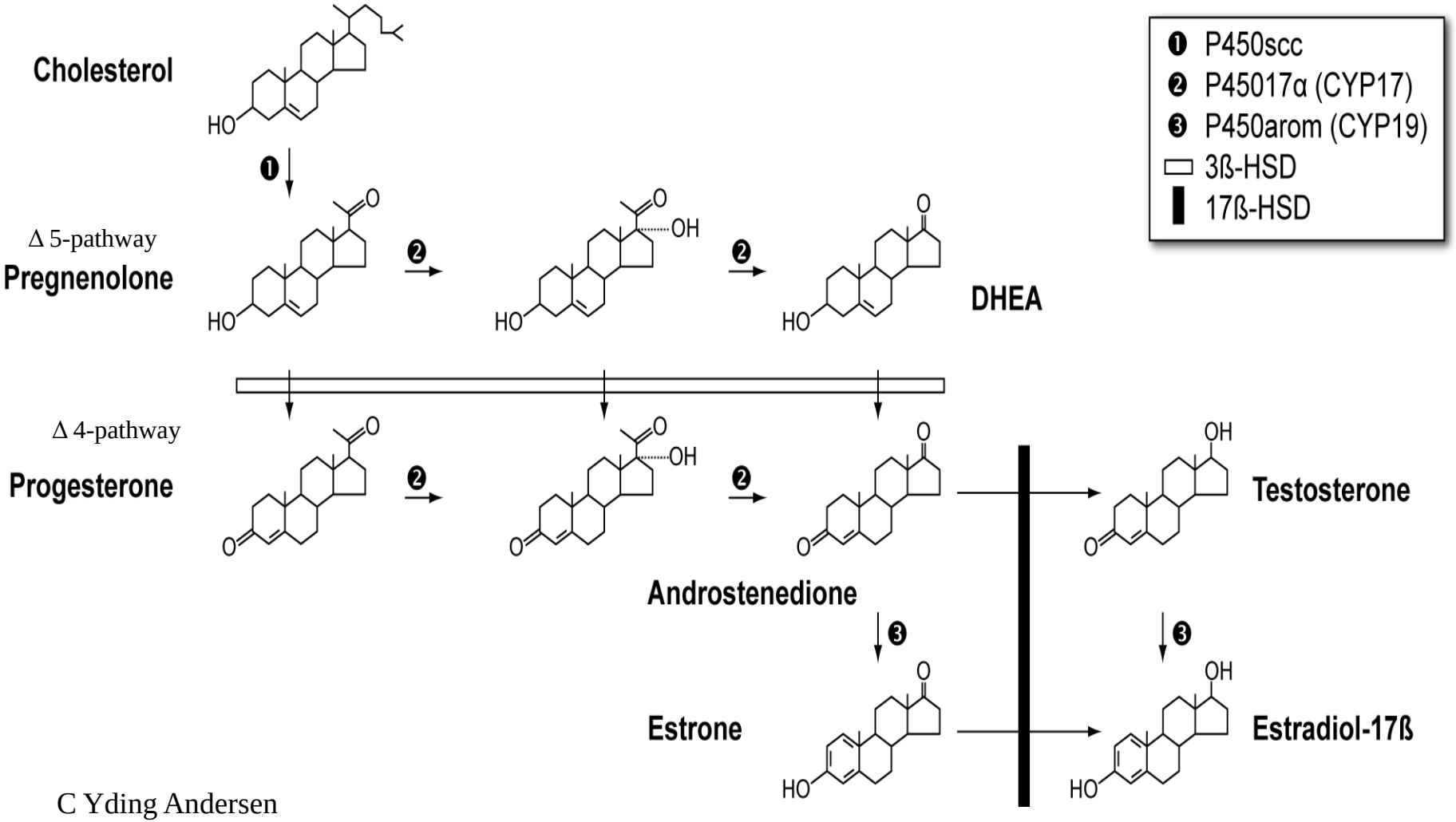
- Studies showing an effect of LH supplementation in subgroups
- Hypotheses as to the effect of LH supplementation in subgroups
- The issue of late follicular phase progesterone rise

# Natural menstrual cycle




Speroff L et al. 5th Edition

# How is follicular progesterone production regulated during controlled ovarian stimulation?




C Yding Andersen

# Fiction…

•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

# Facts…

• 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

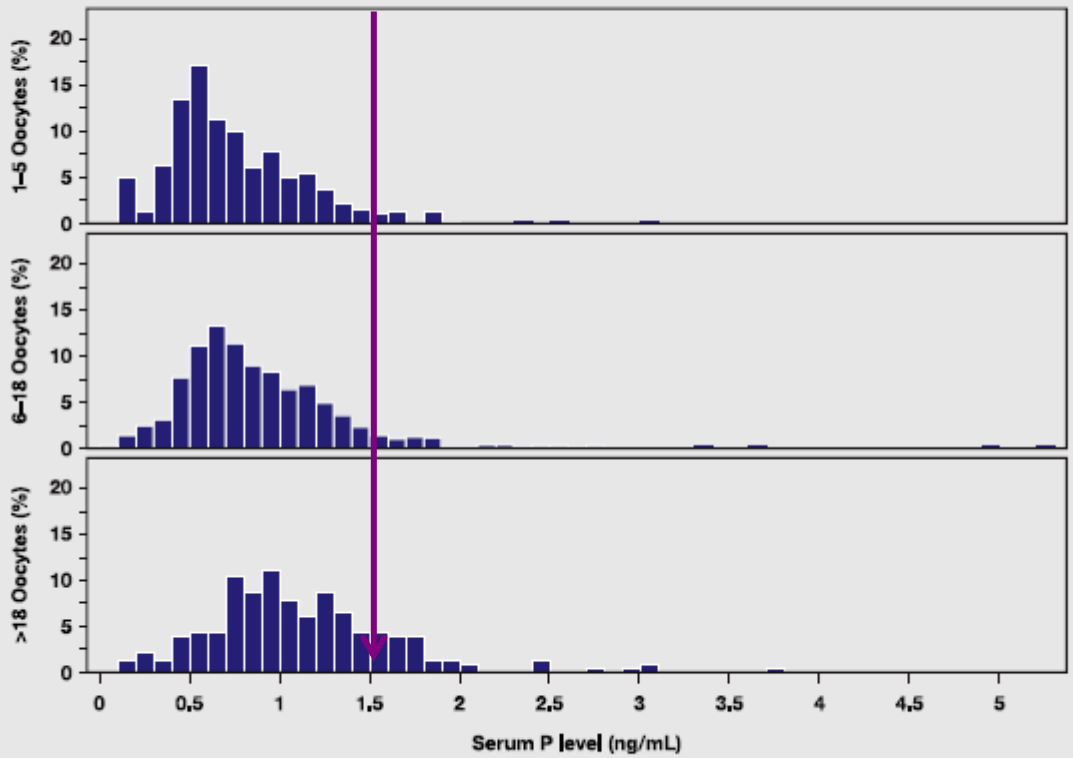
• The **main driver** of the production of P4 in the follicular compartment is an increase in **FSH and LH or hCG**

• 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

# Griesinger et al., 6 studies - 1866 cycles

**FIGURE 1**




Frequency distribution of serum P levels on the day of hCG administration for women with low ovarian response (<6 oocytes), normal ovarian response (6–18 oocytes), and high ovarian response (>18 oocytes).

*Griesinger. Elevated P and ongoing pregnancy. Fertil Steril 2013.*

Fertil Steril 2013

Incidence 4.5% in low responder – 19.0 % in high responder

Update, Vol.19, No.5 pp. 433–457, 2013
on on July 4, 2013 doi:10.1093/humupd/dmt014

# Progesterone 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 %)

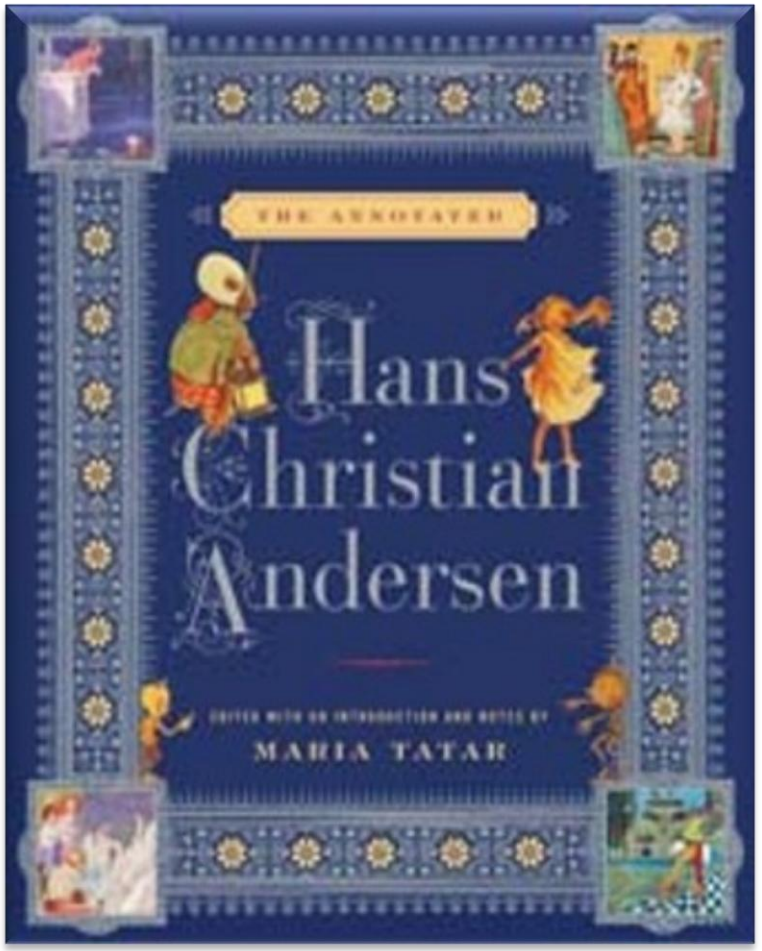
Is this relevant for daily clinical practise?

# Late follicular phase progesterone rise 2014

- Late follicular phase progesterone rise and its consequenses –

  A fairy tale

Lets move on…




Thank You for Your attention
peter.humaidan@midt.rm.dk