Current opinion on use of luteinizing hormone supplementation in assisted reproduction therapy: an Asian perspective

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Introduction

- ovarian stimulation: daily injections of r-FSH to induce multiple follicle growth in the ovaries.
- combined with daily injections of a GnRH agonist or antagonist to prevent a premature LH surge
  - agonists → pituitary down-regulation
  - Antagonist → block of GnRH receptors
- Some assisted reproduction practitioners deem add-back LH to be unnecessary, justifying that the small amounts of LH present after down-regulation are sufficient to sustain theca and granulosa cell stimulation.

- The majority of the data published on the benefits of adding LH to ovarian stimulation do not originate in Asia.
- The average age of couples seeking assisted reproduction technology is rising in Asian.
- A survey of the group's members indicated that > 50% of their patients are above age 35.

- a review of the recent studies was undertaken on the use of LH in ART, including some non-randomized controlled trials (RCT) omitted from the Cochrane review.
- The current published studies comparing r-HFSH VS. r-HLH in ovarian stimulation → too heterogenous or too few in number to conduct an adequate meta-analysis.
- It was estimated that to detect a 5% difference in clinical pregnancy with 90% power would require more than 3000 patients.

Physiology

- The LH and FSH → follicular growth and ovulation.
- These two gonadotrophins are active in the final weeks of the development of a mature surviving oocyte
- The ovarian theca and granulosa cells are the principal sites of LH bioactivity, although LH receptors are also present in extra-gonadal sites such as the uterus.
- According to the two-cell, two-gonadotrophin theory:
  - only FSH is essential for triggering antral follicle formation and follicular growth
  - LH is essential in the pre-antral stage (follicle size <10 mm) to stimulate secretion of androgens by thecal cells

- This synergism between LH and FSH → develop the subsequent capacity of the follicle to ovulate and luteinize when exposed to the mid-cycle LH surge.
- By around days 7–9 (follicle diameter about 10–12 mm), granulose cells stimulated by the effect of FSH begin developing LH receptors in preparation for the final stages of follicle maturation.
- As such, LH plays an increasingly important role after day 6 in regulating the final stages of oocyte maturation.

- The LH surge (days 13–14) induces resumption of meiosis I in the oocyte → early luteinization of the granulosa and theca cells → initiation of the synthesis of progesterone and the production of prostaglandins within the follicle
- These two substances are essential to allow rupture of the follicular wall and eventual liberation of the oocyte about 38–42 h after the LH surge.

The role of r-HLH and the LH therapeutic window

- LH + FSH → stimulating follicular development in women with severe luteinizing LH and FSH deficiency (endogenous serum LH concentration <1.2 IU/l)
- the ovarian follicle requires a minimal amount of LH for steroidogenesis (<1% of receptors attached by LH).
- excessively high concentrations of LH may actually suppress granulosa aromatase activity and inhibit cell growth → The LH ceiling is dependent on timing of the menstrual cycle but for optimal follicle development, this concentration is typically 1.2 IU/l and 5 IU/l

- In the clinical situation, the LH therapeutic window is best observed in two patient groups where there may be a severe endogenous deficiency of LH.
- Hypogonadotrophic hypogonadism (hypo-hypo) patients are the first and probably the most extensively studied group of patients with an endogenous severe LH and FSH deficiency
  - excellent safety and efficacy and is standard treatment for clinicians
- The second group: patients whose endogenous LH secretion is profoundly suppressed with GnRH analogues (agonists or antagonists) during ovarian stimulation who may develop severe endogenous LH deficiency.
  - The role of r-HLH in this group of patients needs further evaluation.

Severe endogenous LH deficiency

- Women with hypo-hypo have impaired pituitary neuroendocrine function that results in abnormally low LH and FSH concentrations.
- These women do not have sufficient endogenous LH for optimal follicular growth and steroidogenesis when treated with FSH alone → typically benefit from FSH and LH for optimal follicular development
- Studies in hypo-hypo women confirmed: r-HFSH was able to increase follicular growth, but was ineffective in stimulating synthesis of oestradiol (extremely low or near-undetectable endogenous LH)
- LH is physiologically essential for oestradiol synthesis

Severe LH deficiency due to suppression by GnRH analogues in assisted reproduction technology

- Women treated with GnRH analogues (agonists or antagonist) during ovarian stimulation in IVF → severely reduced LH and FSH concentrations due to oversuppression of endogenous LH and FSH pituitary secretion.
- In selected patients whose endogenous LH is low after GnRH agonist treatment → poorer outcomes among those patients who have a lower LH concentration or a sharper fall in LH from baseline concentrations

- A retrospective analysis (n = 200) : normogonadotrophic women with long GnRH agonist protocol IVF cycles and treated with r-HFSH were five times more likely to suffer early pregnancy loss if LH serum concentrations on stimulation day 8 were below 0.5 IU/l ($P < 0.005$) (Westergaard et al., 2000).
- A recent two-treatment arm RCT (Pezzuto et al., 2010): compared r-HFSH versus r-HFSH combined with r-HLH, in a long agonist ART cohort with day-6 LH concentrations <0.5 IU/l.
  - There were no differences between the groups in the number of oocytes retrieved (6.37 ± 2.67 versus 7.32 ± 1.99, respectively)

- a significantly higher number of mature oocytes were obtained from the group receiving r-HLH (136 versus 93, $P < 0.05$) and fertilized oocytes (92% versus 69%, $P < 0.001$).
- Clinical pregnancy rate was 5% for r-HFSH alone compared with 22% with r-HFSH plus r-HLH ($P < 0.05$).

- As described earlier, a certain minimum LH concentration is necessary for adequate thecal cell function and subsequent oestradiol synthesis in the granulosa cells.
- The consequent rise in oestradiol concentration is essential for endometrial proliferation and corpus luteum formation in anticipation of a fertilized oocyte, implantation and embryo development in pregnancy.

- While it may be tempting to supplement all patients with LH to reap its benefits, it is critical to take note of the ceiling effect of LH supplementation.
- Early overexposure of LH in ovarian stimulation → premature follicle luteinization of small follicles and follicular atresia → cycle cancellation due to follicle maturation arrest or to poor-quality oocytes, all of which translates into severely compromised outcomes.
- Table 1 summarizes the impact of LH concentrations within and outside the therapeutic window.

Table 1 Concept of LH therapeutic window.

Below LH threshold (<1.2 IU/l)	*Within LH therapeutic window (1.2–5 IU/l)*	*Above LH ceiling (>5 IU/l)*
Impaired follicular development	Optimal follicular growth and development	LH receptor down-regulation
Inadequate thecal androgen synthesis and hence reduced granulosa aromatization to oestrogen	Full oocyte maturation	Suppression of granulosa cell proliferation
No full oocyte maturation	–	Follicular atresia (non-dominant follicles) Premature luteinization (preovulatory follicle)

Adapted from Balasch and Fábregues (2002) and O'Dea et al. (2008).

Exogenous LH supplementation: molecular and functional differences between recombinant human LH and human menopausal and chorionic gonadotrophins

- Endogenous LH production is pulsatile and occurs in response to the pulsatile release of GnRH from the hypothalamus.
- The most physiological way to maintain LH concentrations is to utilize endogenous LH secretion (a short GnRH agonist or microflare protocol in some of their older or poor-responder patients).
- If an exogenous source of LH is needed, physicians have a choice of either urinary human menopausal gonadotrophin (HMG) or r-HLH.

- r-HLH is analogous to endogenous LH and characterized by high purity, precision of dosing and consistency.
- When administered by subcutaneous injection, r-HLH has a terminal half-life of 24 h *(le Cotonnec et al., 1998)* and exhibits modest accumulation with an accumulation ratio of 1.6 ± 0.8.

Human menopausal and chorionic gonadotrophins

- HMG: a urine-derived preparation containing both FSH and LH, which comprises about 5% of the total protein content.
- different HMG preparations are subject to wide variation in LH quantity and bioactivity and with increased purification, more LH is lost.
- For this reason, human chorionic gonadotrophin (HCG) is often added in an attempt to boost the LH bioactivity to meet the required LH activity range (FSH:LH ratio = 1:1).
- This may result in the end-product having much more HCG than LH activity.

- Analysis of one HMG product (Menopur) showed that the content of HCG was more than 10 times higher than LH *(Baer and Loumaye, 2003).*
- Another analysis *(van de Weijer et al., 2003)* also showed that about 95% of the LH receptor bioactivity in one HMG product (Menopur) was attributed to HCG, with less than 5% contributed by pure LH.
- It should be noted that HCG is not normally present in women except during pregnancy and malignancy.

Functional and molecular differences between HCG and LH
(HCG has higher binding affinity and longer half-life)

- HCG has a higher binding affinity to the LH receptor (approximately 6–8 times greater than LH).
- HCG (subcutaneous injection) exhibits a longer serum half-life (30 h) → significant accumulation over time.

(HCG/HMG may induce LH receptor internalization)

- In rats injected with HCG, the ovaries showed LH receptor down-regulation lasting up to 72 h, just as prolonged GnRH agonist stimulation leads to downregulation *(Menon et al., 2006).*
- Recent study: statistically significantly reduced expression of LH receptor messenger RNA in ovarian granulosa cells in the HMG group (VS. HFSH group) which was associated with altered expression of genes and proteins involved in steroidogenesis in preovulatory granulosa cells
- These studies show that HCG is not equivalent to LH and there are effects at the level of LH receptor internalization, which may explain why there is 'tolerance' or a lack of effect.

Recombinant human LH

- r-HLH has recently become available in many Asian countries.
- high purity, precision of dosing and consistency
- r-HLH (subcutaneous injection) has a terminal half-life of 24 h
- In a RCT with patients with a suboptimal response to stimulation with a long GnRH agonist stimulation protocol that compared adding higher doses of r-HFSH versus adding r-HLH or HMG (Ferraretti et al., 2004).
  - those given r-HLH (n = 54) had higher live-birth rates (40.7%) than those given HMG (18%).
  - The r-HLH group also had higher implantation rates

Exogenous LH supplementation in Asia: consensus and recommendations

- Asian assisted reproduction practitioners make use of both long agonist and antagonist protocols for ovarian stimulation; experience with the former is greater.
- Published literature on the beneficial effects of exogenous LH in patients with previous suboptimal response or low baseline serum LH concentrations is more extensive in long agonist protocols
- Table 2 lists and summarizes the group's consensus recommendations and the supporting studies
- The evidence for addition of r-HLH to r-HFSH in antagonist protocols for ovarian stimulation is still being accumulated and more data from future studies is awaited.

Table 2 Evidence supporting recommendations guiding use of recombinant human (r) in assisted reproduction patient subpopulations.

Variables	Prior poor response	Ongoing suboptimal response	At risk of suboptimal response	
			Age >35 years	Day 6 LH <0.5 IU/L
GnRH long agonist	Lisi et al. (2003) Mochtar et al. (2009) Barrenetxea et al. (2008)	De Placido et al. (2004, 2005) Ferraretti et al. (2004)	Marrs et al. (2004) Humaidan et al. (2010) Matorras et al. (2009)	Pezzuto et al. (2010)
GnRH antagonist	De Placido et al. (2006)	–	Bosch et al. (2011)	–
Consensus	Recommended	Recommended	Recommended	More research needed

GnRH = gonadotrophin-releasing hormone.

Patient subpopulations where there is substantial evidence of a benefit of adding r-HLH in ovarian stimulation (poor responders)

- There is substantial evidence of a benefit of adding r-HLH to women who have a poor response to ovarian stimulation, including:
  - (i) poor response in a previous cycle (Lisi et al., 2003; Mochtar et al., 2009);
  - (ii) a suboptimal ovarian response with suboptimal follicular progression in a current cycle by day 6–8
- These recommendations apply only to the use of r-HLH which is analogous to endogenous LH in terms of bioactivity, purity and consistency and is the most physiological replacement in terms of binding affinity and half-life.

- Currently, there is a lack of consensus in Asia on the definition of a prior poor response.
- This review defines prior poor response as
  - an oocyte count of < 4 from a previous stimulation cycle.
  - r-HFSH dose >3000 IU per completed stimulation cycle (Kailasam et al., 2004)
  - and/or less than 800 pg/ml oestradiol on the day of HCG injection.

- In an ongoing cycle, there is a group of patients who, after using suppressive GnRH analogues (either agonists or antagonists), develop severe LH deficiency and exhibit a suboptimal ovarian follicular response by day 6–8.
- This review defines this suboptimal ovarian response as:
  - (i) having no follicle >10 mm by day 6 (De Placido et al., 2005);
  - (ii) low oestradiol concentration <200 pg/ml by day 6 (Vuong et al., 2004)
  - (iii) poor progression or slowing of follicle growth, i.e., previously 1–2 mm progression/day slowing to less than 2 mm in 3 days
- There is an opportunity in this group of patients to salvage the ongoing cycle through r-HLH supplementation.
- When compared with increasing r-HFSH dose, adding r-HLH on day 8 was associated with a better cumulative implantation rate (14.2 vs 10.5, $P < 0.05$) and cumulative pregnancy rate (37.2 vs 29.3, $P < 0.05$)

- This review recommends the use of LH supplementation in the ongoing cycle for:
  - (i) patients with a history of prior poor response (Lisi et al., 2003; Mochtar et al., 2009);
  - (ii) patients who exhibit a suboptimal response during long agonist ovarian stimulation protocols. (De Placido et al.,2005; Pezzuto et al., 2010).
- There is a possibility to optimize the ovarian response in both these patient groups through the addition of r-HLH.

Patient subpopulations where there is evidence of a benefit of adding r-HLH in ovarian stimulation (at risk of hyporesponse)

- This review concludes → the use of r-HLH adjuvant treatment in the following at-risk patients who have markers suggestive of suboptimal ovarian response:
  - (i) women aged >35 years undergoing ovarian stimulation with long GnRH agonist protocol – three RCT: r-HLH in addition to r-HFSH in ART with long luteal-phase agonist protocols *(Humaidan et al., 2004; Marrs et al., 2004; Matorras et al., 2009);*
  - (ii) women aged >35 years undergoing ovarian stimulation with GnRH antagonist protocol – two RCT : benefit of addition of r-HLH to r-HFSH based stimulation

- Based on these clinical studies and personal clinical experience, this review concurred that adjuvant r-HLH starting on either day 1 of stimulation or day 6–8 may be beneficial in patients older than 35 years in long agonist or antagonist protocol ovarian stimulation.
- Monitoring of the response to add-back LH will depend on clinical tests and equipment available.
- The monitoring of follicular progression, oestradiol concentrations and endometrial thickness as a clinical measure of LH response is suggested.

Patient subpopulations where r-HLH is probably not needed or not shown to improve clinical pregnancy

- Based on a meta-analysis of RCT comparing r-HFSH versus r-HFSH plus r-HLH ovarian stimulation → unselected patients undergoing assisted reproduction technology there is no difference between the two treatments in live-birth rate (odds ratio (OR) 0.92, 95% confidence intervals (CI) 0.65–1.31 → NOT recommend adding LH to unselected patients **(age <35 years).**
- However, it should be noted that the authors also mention that their conclusion should be interpreted with caution as the number of subjects (n = 701) was insufficient o reach statistical significance

Patient subpopulations where r-HLH may be of benefit but further research will be needed to quantify the benefit

- There is interest in the use of biomarkers to identify patients at risk of LH deficiency.
- Some putative biomarkers include:
  - (i) *LH concentrations* either at baseline or midfollicular –one study *(Pezzuto et al., 2010)* shows a benefit in patients with day-6 mid-follicular LH concentrations <0.5 U/l, this finding needs to be corroborated by larger RCT;
  - (ii) *anti-Mu¨llerian hormone (AMH)* or *antral follicle count (AFC)* –a retrospective study (n = 80) in Indonesian women found AMH to be a good predictor of ovarian response in IVF *(Wiweko, 2010).*

- But there are currently no prospective studies in Asian women using either AFC or AMH reliably to predict who may need adjuvant r-HLH treatment
- *Alviggi et al. (2009)* recommends that further research be done in patients at risk of poor ovarian response based on the following biomarkers:
  - (i) AFC <6 in both ovaries;
  - (ii) AMH concentration <1.5 ng/ml
  - (iii) LH polymorphisms

Dose and timing of initiation of r-HLH

- The dose of r-HLH in hypo-hypo patients as stated in the summary of product characteristics for r-HLH is 75 IU combined with 150 IU r-HFSH, i.e. a 2:1 ratio of FSH to LH.
- In patients undergoing ART with prevention of LH surge using GnRH analogues, most of the published studies on the combination of r-HLH and r-HFSH in suboptimal responders used r-HLH doses of 75–150 IU daily combined with r-HFSH doses of 300–375 IU.

- In a study that compared either 75 IU or 150 IU r-HLH with r-HFSH (follitropin a or follitropin b) in suboptimal responders with r-HFSH alone in normal responders, significantly more oocytes were retrieved from the 150 IU r-HLH plus r-HFSH group (De Placido et al., 2004).
- In a patient with suboptimal response, this review suggests initiating 150 IU r-HLH combined with 300 IU r-HFSH on either day 1 or 6 of stimulation.

- The timing of initiation of r-HLH in ovarian stimulation: there is no evidence supporting either day 1 or day 6–8 for starting r-HLH.
- However, in theory there may be a benefit to starting patients on day 1 if a clinician wants to maximize the benefit of increased ovarian androgen production.
- From day 1 → increase circulating androgen concentrations → can act synergistically to promote FSH receptor mRNA expression, follicular development and steroidogenesis (Weil et al., 1999).
- Table 3 summarizes recommendations for LH use in patients on long GnRH agonist ovarian stimulation protocols.

Table 3 Consensus on recommended use of LH in Asian women undergoing long gonadotrophin-releasing hormone agonist protocols.

Patient category	Indication	Supporting citation(s)
Substantial evidence of benefit of r-HLH in addition to r-HFSH	Prior poor responders, defined as oocyte count <4 in previous cycle	Mochtar et al. (2009) and Lisi et al. (2003)
	Mid-follicular (day 6) suboptimal response on long agonist	
	No follicles >10 mm	De Placido et al. (2004, 2005) and Ferraretti et al. (2004)
	Oestradiol <200 pg/ml	
	Endometrial thickness <6 mm	Vuong et al. (2004)
Some evidence of benefit of r-HLH in addition to r-HFSH	Age >35 years started on ovarian stimulation with either the long agonist or antagonist protocol	Long agonist: Humaidan et al. (2004), Marrs et al. (2004) and Astorras et al. (2009) Antagonist: De Placido et al. (2006) and Bosch et al. (2011)
Further research is needed to determine benefit of r-HLH	Biomarkers e.g. variant LH	
	Low baseline serum LH <1.2 IU/l	
	Low antral follicle count	
	Low anti-Müllerian hormone	

r-HFSH = recombinant human FSH; r-HLH = recombinant human LH.

Use of adjuvant r-HLH in poor/suboptimal responders to ovarian stimulation in antagonist protocols

- Most of the above recommendations are based on data drawn mainly from studies using a long agonist protocol.
- There are fewer studies examining the situation with respect to antagonist protocols, although in theory, their use should follow similar recommendations for poor responders as in long agonist protocols.

- In a study of 133 poor responders comparing the GnRH antagonist cetrorelix plus r-HLH to GnRH agonist triptorelin flare-up protocol → greater mean number of metaphase-II oocytes
- A RCT *(Acevedo et al., 2004)* in oocyte donors that compared r-HFSH versus r-HFSH combined with r-HLH in an antagonist protocol → significantly higher :
  - metaphase-II oocyte count (80% versus 71%, $P < 0.05$),
  - fertilization rates (83% versus 71%, $P < 0.05$)
  - grade 1 embryos (17% versus 3%, $P < 0.05$)
  - implantation rates (35% versus 15%, $P < 0.05$)

Conclusions

- The complex interplay of LH and FSH and their complementary actions are critical to optimal follicle development and eventual ovulation.
- A minimum threshold concentration of LH is essential in the mid-follicular phase for steroidogenesis in the thecal cells to ensure adequate oestradiol synthesis within the granulosa cells.
- In the mid-cycle phase, a surge in LH is required for final maturation and follicle rupture and ovulation.
- if the LH surge above a certain ceiling concentration occurs prematurely → liberated oocyte may be immature, of poor quality and hence not conducive to a successful pregnancy.

- The LH therapeutic window is best observed in two patient groups:
  - hypo-hypo patients
  - patients who are profoundly suppressed by down-regulation with either GnRH agonists or antagonists in ART.
- It is now recognized that with GnRH analogue protocols in ovarian stimulation, levels of LH bioactivity in some patients (e.g. age >35) may be reduced to below the threshold, who thereby need adjuvant r-HLH.

- The strongest predictive factor for need of exogenous LH in ART is a prior poor or suboptimal response to ovarian stimulation.
- In the case of Asian women, this is defined as an oocyte count < 4 and this review recommends that these women **should be considered for exogenous LH in the next cycle.**
- Another important group who benefit from adjuvant r-HLH in addition to r-HFSH are women who exhibit suboptimal ovarian response during ovarian stimulation as characterized by:
  - (i) no follicle >10 mm by day 6–8;
  - (ii) low oestradiol (<180 pg/ml) by day 6;
  - (iii) poor progression or slowing of follicle growth, with previously 1–2 mm progression per day slowing to less than 2 mm in 3 days.

- There is increasing evidence that age is an important marker of deficient LH bioactivity in women undergoing ART, with multiple studies showing benefit in women aged above 35 years.
- This review recommended adding 75 IU r-HLH per day in these patients from day 6.
- While there are studies supporting the use of r-HLH in addition to r-HFSH in GnRH antagonist protocols, these are fewer in number.
- This is an area that warrants further research.

Thanks for your attention!