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# Understanding the role of LH: myths and facts

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## Abstract

This review summarizes a series of lectures given at a recent Continuing Medical Education meeting in Hamburg, Germany (May 2007), aiming to understand the role of luteinizing hormone (LH) in follicular development during the natural menstrual cycle and controlled ovarian stimulation. Clinical situations and target groups of patients who might benefit from LH supplementation during their ovarian stimulation were discussed and defined. The lectures updated knowledge on the physiology of LH during the normal menstrual cycle and the role of LH in ovarian stimulation. The concept of the 'LH window' was presented, and the use of LH supplementation in different groups of patients undergoing controlled ovarian stimulation was discussed, including those with advanced age, hypogonadotrophic hypogonadism, pituitary down-regulation and poor response. In addition, the different ways of using LH or human chorionic gonadotrophin supplementation in ovulation induction protocols were described.

***Keywords:*** *folliculogenesis, gonadotrophin, gonadotrophin-releasing hormone analogue, luteinizing hormone*

## Introduction

Prior to ovulation, the ovarian follicle undergoes up to 6 months of development, during which it is exposed to both follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

While primary follicular recruitment and pre-antral development can occur in the absence of gonadotrophins, increasing evidence suggests that follicles may then become sensitive to gonadotrophin concentrations and FSH is required for antrum formation; antral follicles become increasingly sensitive to, and eventually dependent upon, FSH and LH.

Both FSH and LH are glycoproteins comprising a common alpha-subunit and hormone-specific beta-subunit. The heavy glycosylation of these proteins affects their half-life and binding affinity, and, hence, their biological activity. Both gonadotrophins bind to specific receptors situated on target cells: FSH receptors are confined to granulosa cells, while LH receptors are present on the theca cells of all follicles and granulosa of large follicles.

FSH and LH were first recognized as two separate entities by Greep *et al.* (1942), which led to the two-cell–two-gonadotrophin theory (Fevold, 1941; Greep *et al.*, 1942). According to this hypothesis, FSH stimulates follicular development and both LH and FSH are required for oestradiol synthesis: LH binds to receptors in the thecal layer to trigger androgen precursors to move from the theca to the granulosa cells, where, through the FSH-stimulated action of aromatase, they are converted to oestrogen.

Of direct relevance to the clinician is the secondary recruitment phase of folliculogenesis and the selection of ovulatory follicles, as this sequence of events can be modulated through the use of exogenous hormones. In an unstimulated cycle, the most mature healthy follicle in the ovary is selected during a period of 14–18 days between the time of luteal regression and ovulation. Between menstrual cycles, FSH concentrations rise and then decline rapidly, whereas LH is secreted in pulses, rising slightly throughout the luteal phase of the cycle. The model put forward by Baird (1987) for single ovulation proposes that it is only during the intercycle rise in FSH that the threshold level is reached and the 'window of recruitment' is opened to allow projection of the largest, healthiest follicle through for ovulation (Baird, 1987). Follicles that miss this window, by being either too late or too early, become atretic. As FSH alone induces development of ovulatory follicles, the question remains: what role does LH play in follicle development and subsequent selection?

## The concept of an 'LH window'

*Zeev Shoham (Israel)*

During this lecture, Zeev Shoham presented the concept of a threshold and ceiling for LH concentration during the follicular phase and discussed the clinical relevance of these concepts.

Following the original study by Greep *et al.* (1942), Berger and Taymor (1971) showed that pituitary FSH alone did not achieve follicular oestradiol secretion – LH was also essential for ovulation induction (Berger and Taymor, 1971). It is thought that LH synergizes with FSH to support oestrogen production; the speaker reviewed studies for and against this two-cell–two-gonadotrophin hypothesis.

A difference in oestradiol production is seen between gonadotrophin-deficient women treated with human menopausal gonadotrophin (HMG) and those treated with urine-derived human FSH (Couzinet *et al.*, 1988). This difference was the subject of a clinical study by the speaker (Shoham *et al.*, 1991). Patients with hypogonadotrophic hypogonadism (HH) were treated during consecutive cycles, with HMG in the first treatment cycle and purified FSH in the second. Compared with HMG, treatment with purified FSH required significantly more ampoules of drug, but resulted in a significant reduction in the number of leading follicles, serum oestradiol concentrations, endometrial thickness and occurrence of ovulation. Thus, the results were consistent with the two-cell–two-gonadotrophin hypothesis.

Once recombinant human FSH (r-hFSH) became available, its *in vivo* efficacy was examined by treating immature female hypophysectomized rats for 4 days with r-hFSH only or with r-hFSH supplemented with human chorionic gonadotrophin (HCG) (Mannaerts *et al.*, 1991). r-hFSH administered at high dose did not increase plasma oestradiol concentrations. However, when r-hFSH was supplemented with only 0.1 IU/l HCG, a three-fold increase in median plasma oestradiol concentrations was obtained. These findings lend further support to the two-cell–two-gonadotrophin theory, showing that both FSH and LH are required for oestrogen biosynthesis, but also revealing that only very small amounts of LH activity are sufficient to increase oestradiol secretion to measurable plasma concentrations. Results from two studies of women with hypogonadism caused by previous hypophysectomy, isolated gonadotrophin deficiency or Kallmann's syndrome were also consistent with the theory. Results showed that FSH alone induced follicular growth, but the low concentrations of androstenedione and oestradiol indicate the requirement for LH to induce appropriate steroidogenesis (Schoot *et al.*, 1994; Shoham *et al.*, 1993). According to the threshold theory, if serum concentrations of LH fall below a minimum level, oestradiol concentrations will be inadequate for endometrial proliferation and corpus luteum formation.

The optimal dose of LH needed in patients with HH has been addressed in a multicentre study (European Recombinant Human LH Study Group, 1998). Patients with World Health Organization (WHO) type-I anovulation were randomized to receive recombinant human LH (r-hLH) (0, 25, 75, or 225 IU/day), in addition to a fixed dose of r-hFSH (150 IU/day). The two highest doses of LH promoted growth of a larger number of follicles and a significantly thicker endometrium than 25 IU LH or no LH, and were associated with a higher clinical pregnancy rate per cycle. The conclusion that 75 IU r-hLH is sufficient for promoting optimal follicular development was also confirmed by a Spanish group (Burgues, 2001). Several studies suggest that this threshold level of LH equates to a serum concentration of 0.5–1.35 IU/l (Fleming *et al.*, 1998, 2000; O'Dea, 2000; Westergaard *et al.*, 2000, 2001). To summarize, LH-deficient patients would benefit from a dose of 75 IU/day, although it remains questionable whether those experiencing pituitary down-regulation following treatment with gonadotrophin-releasing hormone (GnRH) antagonists need LH supplementation.

The concept of an LH ceiling was put forward (Hillier, 1994) based on the fact that the sudden increase in LH, known as the LH surge, triggers follicular maturation and rupture. Evidence for the effect of a high dose of LH first came from studies in rat and human granulosa cells, which indicated that high-dose LH negatively regulates cell growth while positively regulating steroid synthesis (Overes *et al.*, 1992; Yong *et al.*, 1992). Around the same time several clinical studies showed that high LH concentration during the follicular phase was associated with poor oocyte quality, reduced rate of fertilization, reduced rate of embryo implantation and a high rate of miscarriage (Conway *et al.*, 1989; Homburg *et al.*, 1988; Howles *et al.*, 1986; Punnonen *et al.*, 1988; Regan *et al.*, 1990; Stanger and Yovich, 1985).

Subsequently, studies were undertaken to determine the optimal dose of LH that can maintain the growth of a dominant follicle and cause atresia of secondary follicles. A multicentre study was conducted to see if it is possible to minimize follicular development and thus reduce rates of multiple pregnancy using exogenously administered LH (Loumaye *et al.*, 2003). In patients with HH, LH supplementation decreased the number of both small and large follicles compared with no LH or LH in addition to FSH. In patients with polycystic ovary syndrome (PCOS), who have hypersensitive ovaries, supplementation with 225 IU or 450 IU LH decreased the number of follicles on the day of HCG administration compared with placebo. Therefore, it appears that normal follicular development ceases, leading to atresia, when exogenous r-hLH exposure is 225 IU or more per day in the late phase of development. However, it is

possible that the decreased number of leading follicles reported in this study occurred as a result of FSH withdrawal – it is not known whether there is a role for FSH in the second part of the follicular phase, and, therefore, it was recommended that a minimal amount of FSH should be maintained. Currently, it is not clear when, in terms of follicle size, the high dose of LH should be initiated or whether a higher dose (700 IU or 1500 IU) might improve the rate of atresia in these patients with PCOS.

Data from a multicentre study add further support to the LH ceiling concept (Hugues *et al*., 2005). Results showed that when LH at a dose of 660 IU was administered to women who had an over-response to FSH treatment, the number of patients with one leading follicle increased from 13.3% in the placebo group to 32.1%. Another study achieved similar results when LH activity was provided in the form of low-dose HCG (Filicori *et al*., 2002).

The speaker summarized the current knowledge about the threshold and ceiling LH concentrations by stating that LH in the stimulation protocol is obligatory in patients with HH, with a dose of 75 IU sufficient for promoting optimal follicular development in most cases. In patients who develop multiple follicles, such as those with PCOS, supplementation with 660 IU r-hLH appears to increase the proportion of patients developing a single dominant follicle. However, the speaker was of the opinion that in the majority of normogonadotrophic patients undergoing GnRH down-regulation, FSH is sufficient.

## Use of LH in current clinical practice: European market research data

*Michael Ludwig (Germany)*

Michael Ludwig presented new market research data from a study performed to understand the use of gonadotrophin stimulation protocols in European clinical practice. Physicians in France, Germany, Italy and Spain were asked their opinions during 1-hour, face-to-face interviews. Participating physicians were fertility or obstetrics/gynaecology specialists who had been qualified for 3–30 years, who treated at least 10 patients in an average month, and who had experience with both the follitropin alfa pre-filled pen and the follitropin beta pre-filled pen, and were not affiliated with any pharmaceutical company. Each physician completed four patient case records, based on patients who had recently received a cycle of injectable gonadotrophins. At the interview, physicians were asked for their current treatment decisions, and for their decisions based on current and future gonadotrophin availability. It is thought that by asking each physician to assess a patient and decision with which they are familiar, their stated behaviour is more likely to accurately predict future behaviour (Ajzen and Fishbein, 1977).

A total of 180 physicians participated, 45 from each country, and therefore 720 patient case records were completed. When asked about their current practice, 36.0% of the physicians prescribed the follitropin alfa pre-filled pen; 31.8% prescribed the follitropin beta pen; 15.5% prescribed r-hFSH in combination with urinary-HMG (u-HMG); 8.8% prescribed r-hFSH combined with r-hLH, and 7.9% used other gonadotrophins or formulations. Thus, approximately one-quarter of existing cycles involved addition of LH activity, either as r-hLH or u-HMG. Most physicians use an FSH:LH ratio of 2:1 (37% patients) reflecting the common practice of adding 75 IU to 150 IU FSH.

One analysis separated prescribing practice according to the type of cycle and the mean age of the patients treated with these preparations. The follitropin beta pre-filled pen was generally administered to the youngest patients (mean age 33.5 years), of whom about 30% were undergoing ovulation induction, while more than 50% of patients prescribed the follitropin alpha pre-filled pen were undergoing assisted reproductive therapy. The use of HMG was generally reserved for slightly older patients (mean age ~35 years) and r-hLH for the oldest patients (mean age ~36 years). However, when offered a fixed combination r-hFSH + r-hLH (2:1) product, physicians indicated that they would use this for approximately 20% of all patients, as an alternative to r-hLH or HMG.

## Does LH play a role? Defining subgroups

*Peter Humaidan (Denmark)*

According to the two-cell–two-gonadotrophin theory (Fevold, 1941), both FSH and LH are required for normal folliculogenesis in humans. Peter Humaidan began his lecture with an overview of the changes in endogenous LH concentrations in long-protocol agonist pituitary down-regulation cycles compared with a spontaneous cycle (Westergaard *et al*., 1998). In a normal cycle, LH concentrations rarely fall below 2 IU/l in the follicular phase, normally ranging from 2 to 5 IU/l. Conversely, in a down-regulated cycle, LH concentrations are already extremely low at stimulation day 1 (1.68 IU/l), and then continue to decrease, falling below the level at which HH is characterized.

There is general agreement that LH supplementation is mandatory in patients with HH, but whether it is necessary for normogonadotrophic women after down-regulation by a long-agonist protocol is still the subject of debate. Such conflict arises because studies are not comparable, with differences in the type, dose, mode of administration and duration of down-regulation, differences in the dose, source, starting day and duration of LH supplementation, and variation in the type of assay used to assess LH activity. Several studies that suggested a role for exogenous LH supplementation in patients with profoundly suppressed endogenous LH (Fleming *et al*., 1996, 2000; Westergaard *et al*., 1996, 2000) disagree with studies showing that patients with high endogenous LH concentrations after down-regulation responded poorly to stimulation (Humaidan *et al*., 2002, 2004).

The most recent of these studies was described during this lecture (Humaidan *et al*., 2004) following the same long-agonist down-regulation protocol as previous studies (Westergaard *et al*., 2000) in an attempt to provide comparable results. On day 8 of stimulation patients were randomized to r-hFSH alone or supplementation with r-hLH in a ratio of 2:1. Results showed a non-significant difference in clinical pregnancy rate of 6% in favour of r-hLH. When patients were divided into four

subgroups on the basis of LH supplementation status and their age, using the same cut-off as a prior study (<35 years, ≥35 years) (Marrs *et al*., 2004), it was found that non-supplemented patients 35 years or older required significantly more FSH, and had significantly lower implantation and pregnancy rates compared with the supplemented patients of the same age or the non-supplemented patients who were under 35 years. This implies that LH supplementation would be beneficial in these patients. Division of patients into three groups according to their endogenous LH concentration on stimulation day 8 (≤1.20 IU/l, 1.21–1.98 IU/l, ≥1.99 IU/l) confirmed that patients with higher endogenous LH concentrations had a reduced requirement for FSH. There was a non-significant tendency for a lower pregnancy rate in the group with highest LH concentrations on day 8 who did not receive LH supplementation compared with those who did, and this was also accompanied by a significant decrease in implantation rate. In this intervention study, the implantation rate of 20% in this group was similarly low compared with that obtained in the category of patients with elevated endogenous LH concentrations in the speaker's previous non-intervention study (23%) (Humaidan *et al*., 2002). Therefore, the results indicate that LH supplementation provides no general benefit to profoundly suppressed patients, but that patients aged 35 years or more, or those who have endogenous LH concentrations ≥1.99 IU/l on day 8 after GnRH agonist down-regulation, would benefit.

Additional subgroups of IVF patients have been investigated. Normogonadotrophic women with an initially inadequate response to r-hFSH were found to have lower total dose of FSH, increased oestradiol concentrations on the day of HCG administration, increased number of oocytes, and an increased pregnancy rate when supplemented with r-hLH compared with those who were not supplemented (Ferraretti *et al*., 2004), and it has been suggested that a daily dose of 150 IU/l r-hLH might be the most beneficial in these patients (De Placido *et al*., 2004). Recently, a small study added to this evidence by showing a lower rate of apoptosis in cumulus cells following r-hLH supplementation compared with control, and concluded that supplementation with LH improves the chromatin quality of cumulus cells involved in the control of oocyte maturation (Ruvolo *et al*., 2007). Thus, at present, the three subgroups of patients who could benefit from LH supplementation after GnRH analogue down-regulation are patients aged 35 years or more, patients with a suboptimal response to FSH alone, and this intriguing group of patients with high LH concentrations after down-regulation.

Similarities between the subgroups likely to benefit from LH supplementation were evaluated by first considering the age-related changes that occur in the ovary. With increasing age, the androgen secretory capacity of thecal cells is reduced (Piltonen *et al*., 2003) and there are decreasing numbers of functional LH receptors (Vihko *et al*., 1996). Moreover, the bioactivity of LH decreases with age, while the immunoreactivity is unchanged, making it difficult to measure (Marrama *et al*., 1984; Mitchell *et al*., 1995). Finally, LH activity is enhanced locally by paracrine activities, but with increasing age, ovarian paracrine activity decreases (Hurwitz and Santoro, 2004). It could be postulated that these two subgroups, with initial low response and high endogenous LH concentrations, may consist of patients with polymorphic variants resulting in altered bioactivity of the LH molecule (Huhtaniemi *et al*., 1999; Jiang *et al*., 1999; Ropelato *et al*., 1999). Alternatively, in these patients the endogenous LH activity falls below a critical threshold value during down-regulation. The situation may be clarified by further investigations into the dynamic changes in LH concentration during down-regulation.

It is clear that more work is needed to ascertain the full beneficial effect of LH and the developing follicle's precise requirement for LH. The speaker emphasized the need to perform extended studies in subgroups of patients to confirm previous observations and to find the optimal dose, time, and frequency of LH activity administration.

## Serum concentrations of LH in individual patients as predictors of ovarian response – the hypo–hypo lesson

*Lars Westergaard (Denmark)*

Lars Westergaard reviewed the evidence for a lower LH threshold in addition to the upper threshold. In women with gonadotrophin deficiency, stimulation with FSH alone leads to the development of pre-ovulatory follicles and fertilization, but also low ovarian oestradiol production and no live births, thus confirming the two-cell–two-gonadotrophin theory that LH is needed for normal follicular development (Couzinet *et al*., 1988; Schoot *et al*., 1994; Shoham *et al*., 1991).

The European Recombinant Human LH study investigated the precise concentrations of LH needed for normal follicular growth in women with WHO I anovulation in order to define the lower threshold and upper ceiling (European Recombinant Human LH Study Group, 1998). There was a significant correlation between the dose of LH administered and oestradiol production. However, in all but three patients (31/34), the serum LH concentration remained below the level that characterizes HH (1.2 IU/l), thus confirming the short half-life of LH. Only patients randomized to 75 IU or 225 IU LH achieved conception. While a trend for a lower number of follicles in the 225 IU group compared with the 75 IU group suggested that the 'ceiling' level of LH had been exceeded, the accompanying exponential increase in oestradiol concentrations following 225 IU compared with 75 IU did not confirm this. When the effect on serum oestradiol concentration was looked at as a function of follicles ≥15 mm diameter on the day of HCG administration, there was no oestradiol production in the groups of patients with no LH, or 25 IU LH, but production doubled in the 225 IU group compared with the 75 IU group. Again, this refutes the theory that the follicle's capacity to produce oestradiol is saturated by 225 IU. In summary, this study found that 75 IU LH is effective in promoting optimal follicular development in the majority of women with HH, with a minority requiring up to 225 IU.

In contrast to women with HH, the level of circulating LH required for optimal follicular development, steroidogenesis and oocyte maturation in pituitary down-regulated normogonadotrophic women is unclear. Evidence for a lower

LH threshold in normogonadotrophic women after pituitary down-regulation with GnRH agonists was discussed. While the LH suppression following a long-protocol GnRH treatment is profound, does it affect the reproductive outcome? Initial work seemed to suggest that resting concentrations of LH may be adequate for normal follicular maturation (Chappel and Howles, 1991), with good pregnancy rates obtained following long-protocol pituitary suppression (Out *et al.*, 1995). However, more recent studies advocate a need for endogenous LH supplementation to increase serum LH above a lower threshold. There is evidence that oestradiol production is reduced when mid-follicular serum LH concentrations are below 1 IU/l (Fleming *et al.*, 1996; Westergaard *et al.*, 1996), and also that severely depressed mid-follicular serum LH concentrations are associated with poorer reproductive outcome (De Placido *et al.*, 2000; Fanchin *et al.*, 2001; Fleming *et al.*, 1998; Tesarik and Mendoza, 2002; Westergaard *et al.*, 2000). The retrospective analysis performed by Westergaard *et al.* using data from 200 normogonadotrophic women found that 50% of patients had severely suppressed LH concentrations (<0.5 IU/l) on stimulation day 8 (Westergaard *et al.*, 2000). There was no difference in the number of live births in these patients compared with those who had serum LH concentration >0.5 IU/l, when given as proportion of started cycles. However, when the number of live births was presented as a proportion of positive pregnancy tests, there were significantly fewer live births (52% versus 77%; $P < 0.05$) in the group with low serum LH concentration, due to a significantly increased rate of early pregnancy loss (45% versus 9%; $P < 0.005$). Such results continue to generate much debate.

The treatment of normogonadotrophic women with a GnRH antagonist causes a steep drop in serum LH concentrations, and several studies have looked in more detail at the effect this has on outcome. Data from the ganirelix dose-finding study were divided into two groups on the basis of LH concentration on the day of HCG administration: less than 1.0 IU/l or greater than or equal to 1.0 IU/l. A significantly increased rate of early pregnancy loss (43%) in the group with low LH concentrations was observed, compared with the higher LH group (10%; $P < 0.0005$). This would imply that GnRH antagonist treatment could suppress endogenous LH concentrations below a clinically meaningful level. However, results from later studies do not confirm this hypothesis. A recent systematic review analysed studies comparing endogenous LH concentrations in normogonadotrophic women, including studies that reported the ongoing pregnancy rate (>12 weeks) or the delivery rate as primary outcome measures. Four studies that used a long mid-luteal phase agonist protocol (Balasch *et al.*, 2001; Esposito *et al.*, 2001; Humaidan *et al.*, 2002; Westergaard *et al.*, 2000) and two studies using an agonist protocol (Kolibianakis *et al.*, 2004; Merviel *et al.*, 2004) were analysed. There was no association between LH below the threshold and outcome measure in any of the studies, with one exception, which found a significantly higher ongoing pregnancy rate in the group with LH concentrations below the threshold (Kolibianakis *et al.*, 2004). As a consequence, a lower LH concentration threshold has not yet been defined in normogonadotrophic women subjected to pituitary suppression with GnRH analogue before ovarian stimulation with FSH. It seems, however, that co-administration of preparations with LH activity (i.e. r-hLH, HCG or HMG) to standard GnRH plus FSH protocols does not affect reproductive outcome in most normogonadotrophic women (van Wely *et al.*, 2003, Andersen *et al.*, 2006).

The speaker speculated on the apparent discrepancy between HH and normogonadotrophic women with respect to their response to lower LH concentrations. The hypogonadotrophic state of much shorter duration in normogonadotrophic women (only 2–3 weeks) suggests that normal LH concentrations in the cycle prior to pituitary suppression act to 'prime' small antral follicles, making them less sensitive to later drops in circulating LH, whereas in women with HH the follicles have been deprived throughout their lifetime. However, it was also noted that this mechanism might work less well, perhaps due to mutations in the receptor, in subgroups of normogonadotrophic women, not identifiable by serum LH concentrations, who would benefit from co-administration of hormone preparations containing LH activity after GnRH analogue down-regulation. These subgroups could include patients over the age of 35 years or patients who are initially low responders (De Placido *et al.*, 2001; Ferraretti *et al.*, 2004; Humaidan *et al.*, 2004; Marrs *et al.*, 2004). More investigations are needed to clarify the situation, particularly in the over-35-years age group.

## LH and LH-like activity supplementation in GnRH agonist and antagonist cycles

*Jean-Noël Hugues (France)*

Jean-Noël Hugues began by presenting the most recent meta-analyses addressing the effect of r-hLH supplementation in GnRH analogue cycles. A new meta-analysis by Kolibianakis *et al.* included five studies using agonist down-regulation, and two that used antagonist down-regulation, with the primary end-point as the live birth rate per randomized patient (Kolibianakis *et al.*, 2007). In this large series, it was observed that there was a difference of 2.3% in favour of treatment with FSH alone compared with FSH supplemented by LH. The most recent Cochrane review includes 14 studies, of which 11 used an agonist protocol to control the LH surge; in total 2396 cycles are included (Mochtar *et al.*, 2007). However, the speaker cautioned that among these cycles are patients with a poor response, or where a flare-up protocol was used, as well as results that have only been published as abstracts. The heterogeneity between clinical trials is so large that the difference between FSH and FSH/LH is not significant if a randomized effect model was used for pooling the data. There was a trend for a decreased miscarriage rate in patients supplemented with LH, which became borderline significant if the cycle with a flare-up protocol was excluded. Baruffi *et al.* (2007) analysed results from five studies looking at the effect of LH supplementation in GnRH antagonist cycles. The only differences observed were a significantly increased oestradiol concentration on the day of HCG administration and a higher number of mature oocytes in patients who had LH supplementation (Baruffi *et al.*, 2007). In conclusion, meta-analyses have not shown any clinical beneficial effect of adding LH in an unselected patient population, although in cycles with GnRH agonists there is a trend for lower spontaneous miscarriage, and in cycles with GnRH antagonists higher oestradiol concentrations have been observed.

The drawbacks of meta-analyses have been discussed widely. Studies included in these meta-analyses vary in several crucial ways. They may use either an unselected population or a subgroup of older (>35 years or >38 years) patients or low or poor responders. If using a subgroup of low or poor responders, their status may be demonstrated or presumed; the criteria used to define poor response are significantly different between studies. Stimulation regimens vary widely according to GnRH analogue protocol used: agonist or antagonist, daily or long-acting formulation, depot or intranasal preparation. Variation is also introduced by the different starting doses of FSH and ensuing dose adjustment. Furthermore, the LH supplementation regimen may differ in dose and time of first administration. Such variance between trials included in the meta-analyses is compounded by the methodology used for the meta-analysis itself; for example, the exclusion criteria employed are essential. In order to detect a 5% difference in live birth rate, 2504 cycles would need to be included; however, only one analysis reached this size (Mochtar *et al*., 2007). Thus, large randomized trials are needed to estimate more precisely the difference between treated groups and subsequently to identify subgroups of patients who would benefit from LH supplementation.

Results of a prospective, randomized, multicentre study looking at LH supplementation following agonist down-regulation were presented at the 2006 annual meeting of the European Society of Human Reproduction and Embryology (ESHRE) (Nyboe Andersen *et al*., 2006). Doses of r-hFSH and r-hLH were individualized in an age-dependent manner. Compared with FSH alone, supplementation with LH resulted in a significant and dose-dependent increase in serum LH concentrations on the day of HCG administration. There were no significant differences between the two groups with respect to ovarian parameters. The large sample size of this study allowed a subanalysis to indicate which patients are more likely to benefit from the addition of LH. Firstly, by analysing ongoing pregnancy according to serum LH, it was observed that patients with low serum LH concentrations had significantly lower pregnancy rates with FSH alone, but this difference was eliminated by supplementation with r-hLH. This finding provides some indirect evidence that in patients with low concentrations of serum LH, the addition of LH might be clinically beneficial. Secondly, by analysing ongoing pregnancy according to patient age, with a cut-off of 35 years, no significant difference in pregnancy rates was observed. This is in contrast to previous studies which showed that LH supplementation is beneficial for patients over the age of 35 years (Humaidan *et al*., 2004; Marrs *et al*., 2004).

The question of poor or low ovarian response was discussed. The meta-analysis by Mochtar showed a trend for LH supplementation in poorly responsive patients (Mochtar *et al*., 2007). However, the rationale behind LH supplementation in poor responders is not very clear. A novel approach to investigate the effects of r-hLH supplementation in low-responder patients undergoing ovarian stimulation with r-hFSH used the rate of apoptosis in cumulus cells as an indicator of oocyte quality (Ruvolo *et al*., 2007). There was a trend for both pregnancy and implantation rates to be higher in the r-hLH group, but this did not reach statistical significance. The increase in pregnancy rate correlated with a higher apoptosis rate in cumulus cells in the control group than in the r-hLH group. This study suggests that supplementation with r-hLH might protect the oocyte from the risk of apoptosis in older patients.

One of the most challenging issues remains the optimal timing of LH-like activity supplementation. All studies to date have supplemented LH during the beginning or middle of FSH stimulation, to increase oestrogen synthesis and reduce follicular growth. However, another option is to give LH in the early stages of folliculogenesis prior to FSH stimulation, and this possibility was raised by the speaker. In this phase, LH supplementation increases androgen production by theca cells and stimulates follicular recruitment. Evidence comes from work in primates: in one study, Hillier *et al*. showed that androgens are able to enhance FSH-induced granulosa cell gene expression, particularly aromatase activity (Hillier and Miro, 1993); in another study, androgen given as either a high dose for 3 days or a low dose for 10 days stimulated the early stages of follicular growth (Vendola *et al*., 1998). Therefore, it is clear that increasing locally produced androgen concentrations stimulates growth of small follicles, and studies have shown that use of aromatase inhibitors in GnRH antagonist protocols increases the level of intrafollicular androgens and improves clinical outcomes (Garcia-Velasco *et al*., 2005; Lossl *et al*., 2006). Subsequently, the concept of 'LH priming' prior to FSH stimulation to stimulate growth of small antral follicles and to improve ovarian responsiveness to FSH has been assessed recently (Fleming *et al*., 2006). Preliminary data showed that priming with 300 IU r-hLH for 7 days might increase the number of small antral follicles and the number of fertilized oocytes. These data emphasize the need for additional clinical trials to investigate whether a long-term exposure to LH-like activity may be effective at improving the overall process of folliculogenesis.

## The case of poor and low responders and the use of exogenous LH supplementation

*Ernesto Bosch (Spain)*

Ernesto Bosch's lecture focused on patients with low and poor response to gonadotrophin stimulation. A low response is a quantitative concept, which could be defined as a lower than average number of oocytes, or alternatively as the number of oocytes at which the chance of pregnancy starts to decline, and is directly related to age. A poor response is a qualitative concept that can be observed when ongoing pregnancy rate data are grouped according to age; a significant trend for decreasing pregnancy rate is seen, despite a reasonable number of oocytes (at least eight). So, although the response from these older patients could not be low in a quantitative sense, their response is qualitatively poor.

The process of reproductive ageing is determined by follicular depletion. Chronological age is a poor indicator of reproductive age because much variation exists between individuals (Soules *et al*., 2001). Premature ovarian ageing is, therefore, difficult to predict. As the ovary ages, fertility declines and the rate of spontaneous abortions increases. Comparison of live birth rates shows an age-related decline in outcome when patients have their own eggs, whereas outcome in patients with donor

eggs remains relatively constant with increasing age. This demonstrates that poor outcome relates to the oocyte rather than uterine factors. Therefore, the process of ovarian ageing has been termed the 'oopause'. The oopause is characterized by low and poor response to gonadotrophin stimulation, inability of the oocyte to re-enter meiosis at metaphase I, lower fertilization and cleavage rates, reduced embryo development and increased risk of aneuploidy.

In addition to clinical signs, endocrinological changes are also involved in the ageing ovary. A subtle rise in the FSH concentration during follicular phase unaccompanied by a rise in LH concentrations is the most consistent finding (Klein *et al*., 1996). It is also known that serum androgen concentrations decline with age: a small study showed that 24-h testosterone concentrations decrease with age (Zumoff *et al*., 1995), and a larger study showed clear decreases (ranging from 49% to 77%) in concentrations of several androgens, which were not transporter-dependent (Davison *et al*., 2005). Such changes are also apparent in stimulated cycles. Welt *et al*. show that, in response to r-hFSH stimulation, oestradiol concentrations increase similarly across the follicular phase, whereas androstenedione concentrations are lower in older women compared with younger women (Welt *et al*., 2006). This suggests that the dynamics of the ageing ovary are different under stimulation compared with the younger ovary, and therefore the combination of gonadotrophins needed for stimulation in older patients should be different from those used for patients in the early and peak reproductive stages.

Several studies have shown a benefit from LH supplementation in patients needing high doses of FSH (Lisi *et al*., 2001; Lisi *et al*., 2002) or in those presenting hypo-responsiveness to FSH in terms of follicular development (De Placido *et al*., 2004; De Placido *et al*., 2005; Ferraretti *et al*., 2004). However, these patients are difficult to identify before starting stimulation. The ovarian response of these patients is not predicted by serum LH concentrations on day 1 of stimulation (Bjercke *et al*., 2005), and results regarding the predictive value of chronological age are contradictory (Fabregues *et al*., 2006; Marrs *et al*., 2004).

The effect of different amounts of r-hLH in oocyte donors undergoing controlled ovarian hyperstimulation on hormonal profile, oocyte and embryo quality, and cycle outcome was investigated in a prospective randomized trial (Bosch *et al*., 2006). Three groups received the same total amount of gonadotrophins, either 300 IU r-hFSH, 225 IU r-hFSH and 75 IU r-hLH, or 150 IU r-hFSH and 150 IU r-hLH. No differences were observed among groups for any of the serum hormone determinations, except for FSH concentrations, which were significantly higher in the r-hFSH-only group. In addition, a trend for higher progesterone concentrations was noted in this group. In the follicular fluid, oestradiol, testosterone and androstenedione concentrations were highest in follicles from the group of patients who had received the highest amount of r-hLH. Therefore, the effect of LH supplementation is seen at the follicular level, rather than in the serum. Interestingly, two studies have shown a clinical benefit of providing increased androgenization prior to stimulation in patients with a history of low or poor response, either by using an aromatase inhibitor (Garcia-Velasco *et al*., 2005) or by pretreatment with testosterone 5 days before FSH stimulation (Balasch *et al*., 2006).

Finally, the speaker presented his own data, which show improved cycle outcomes in women with low response or who are over 38 years of age when supplemented with LH-like activity (data not published). In the group undergoing long-protocol down-regulation, this improvement was seen when either HMG or r-hLH were used as the source of LH-like activity. However, in the group down-regulated with antagonists, this benefit was only seen when the LH-like activity was provided by r-hLH, not HMG.

In conclusion, LH supplementation appears to be appropriate for poor and low responders to restore the follicular milieu and oocyte competence, and to improve cycle outcome. Concerning further studies, the speaker recommended looking at LH, supplementation according to basal androgen concentrations.

## Overall conclusion

By summarizing current knowledge about the role of LH activity in natural and stimulated cycles, these lectures have highlighted the potential benefit of exogenous LH supplementation during ovarian stimulation, not only in patients with HH but also in certain subgroups of normogonadotrophic patients: patients >35 years of age, patients with initial low or poor response, patients with deeply suppressed LH concentrations after pituitary down-regulation, or patients with PCOS who have elevated concentrations of LH which does not appear to be fully bioactive.

However, while the basic biochemical principles of LH action have been elucidated and the LH therapeutic window described, the mechanism underlying the LH requirement in these different patient subgroups is not yet known. A possible hypothesis centres on biological ageing of the ovary, perhaps involving decreased gonadotrophin receptor sensitivity mediated via accumulation of mutations.

It is hoped that this review will encourage further carefully-designed studies, both clinical and molecular, to clarify the use of LH in daily stimulation protocols in terms of optimal timing, dose and frequency of supplementation, allowing more individually tailored treatment regimens, which will subsequently lead to improved cycle outcomes.

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