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Commentary

LH in the follicular phase: neither too high nor too low

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Abstract

The role of LH in the natural menstrual cycle is not disputed. However, there are a variety of opinions regarding the potential role of exogenous LH in ovulation induction and whether it is actually needed. Recent years have seen renewed interest in this issue for several reasons. First, ovulation-inducing drugs are increasingly being administered to normally ovulating women. Second, recombinant human FSH products completely devoid of LH activity are now available. Third, gonadotrophin-releasing hormone (GnRH) analogues (agonists and antagonists) prevent the untimely LH surge but also suppress endogenous LH activity during the follicular phase. This review analyses whether or not all patients need LH for follicular growth stimulation and new opportunities for improved treatment as a result of the availability of recombinant human LH both in patients with ovulatory disorders (World Health Organization (WHO) groups I and II anovulatory patients) and those undergoing multiple follicular development for assisted reproduction.

Keywords: *folliculogenesis, GnRH analogues, IVF, LH, ovulation induction*

Introduction

The role of LH in the natural menstrual cycle is not disputed. Basic science and experimental and clinical data have shown that LH is strictly necessary for normal follicular development and oocyte maturation (Balasch and Fábregues, 2002). However, the optimal ratio of FSH-to-LH activity during ovarian stimulation has been a matter of controversy since the very early days of gonadotrophin therapy (Jacobson and Marshall, 1969; Louwerens, 1969). Urinary human menopausal gonadotrophin (HMG) was originally the only preparation available for clinical use, and it has been widely used for ovarian stimulation since its introduction in the early 1970s. The use of HMG [theoretically containing similar amounts (75 IU) of FSH and LH] has been based on classic experiments in hypophysectomized rats treated with more or less pure forms of FSH and LH obtained from animal pituitary glands, and suggesting that both hormones were necessary to stimulate pre-ovulatory follicular development and synthesis of oestrogen (Fevold, 1941; Greep *et al*., 1942; Short, 1962). Thus it has been generally accepted that satisfactory clinical results may be obtained irrespective of the LH content of the preparation, provided that adequate FSH is administered. This means that clinicians usually relied on the use of urinary gonadotrophin extracts containing FSH with varying amounts of LH, making it difficult to selectively alter the dose of either gonadotrophin, while experimentalists resorted to scarce and expensive pituitary preparations of varying purity (Hillier, 2001). The breakthrough came with the recent availability of recombinant human FSH (rFSH) and LH (rLH), which are truly monohormonal products, and hence provide powerful new tools in experimental endocrinology thus allowing a clear definition of the individual roles of FSH and LH on follicular development in humans (Hillier, 1994, 2001).

Thus, recent years have seen renewed interest in this issue for several reasons. First, ovulation-inducing drugs are increasingly being administered to normally ovulating women. Second, rFSH products completely devoid of LH activity are now available. Third, GnRH analogues prevent the untimely LH surge but also suppress endogenous LH activity during the follicular phase. Thus, while the relative importance of LH in the follicular phase and its role in the stimulation of follicular growth and maturation have not been yet fully elucidated, the possible impact of LH on the outcome of gonadotrophin-stimulated cycles has been widely discussed in the recent literature (Balasch and Fábregues, 2002; Balasch, 2004; Kol, 2005; Humaidan, 2006).

Therefore, as recently stressed (Hugues, 2002), there is a need for further clinical research to establish appropriate clinical criteria for LH supplementation. This report is an attempt to provide further insight into the subject.

The window for LH: the 'threshold' dose and 'ceiling' value concepts

Follicular responsiveness to FSH and LH is developmentally regulated. FSH plays a crucial part in recruitment, selection, and dominance, while LH contributes to dominance, final maturation, and ovulation (Hillier, 1994, 2001; Zeleznik, 2001). There is basic, experimental and clinical evidence unequivocally indicating that ovarian follicles have development-related requirements for stimulation by LH, that is, there is a 'threshold' for LH requirements during folliculogenesis (Hillier, 1994, 2001; Balasch *et al*., 1995; Balasch and Fábregues, 2002). The amount of LH activity actually necessary for normal follicle and oocyte development, however, is not known, but is likely to be very low, since less than 1% of follicular LH receptors need to be occupied in order to elicit a maximal steroidogenic response, and, accordingly, resting concentrations of LH should be sufficient to provide maximal stimulation to theca cells (Chappel and Howles, 1991).

Although LH is essential for oestrogen synthesis and maintenance of follicular dominance, there is clinical evidence that excessive stimulation of the ovaries by LH adversely affects normal pre-ovulatory development. Depending on the stage of development, follicles exposed to inappropriately high concentrations of LH enter atresia or become prematurely luteinized, and oocyte development may be compromised (Chappel and Howles, 1991; Hillier, 1994, 2000; Homburg, 1998; Huirne *et al*., 2005). Thus, developing follicles appear to have finite requirements for stimulation by LH, beyond which normal development ceases. Whereas each follicle has a threshold beyond which it must be stimulated by FSH to initiate pre-ovulatory development, it may also have a 'ceiling' within which it should be stimulated by LH, unless further normal development is terminated; remarkably, mature follicles are more resistant (higher 'ceiling') to LH than immature ones (Hillier, 1993, 1994).

The LH 'ceiling' hypothesis is further supported by two well-known clinical conditions that may be associated with reproductive failure: ovulation induction with clomifene citrate in the anovulatory patient, and the use of the short protocol with GnRH agonists in IVF. Clomifene is used in World Health Organization (WHO) group II anovulatory patients (mainly polycystic ovarian syndrome (PCOS)). The main mode of action of clomifene is to boost FSH in the early to midfollicular phase but, unfortunately, it also raises LH concentrations at this apparently critical stage and, mainly for those patients who already have a high baseline concentration, the additional discharge of LH may prejudice their chances of conceiving. Both the lack of conception in the face of an apparent ovulatory pattern, and an increased risk of miscarriage have been reported with clomifene citrate. Inappropriate LH action interfering with follicular and oocyte maturation would explain these adverse reproductive effects (Homburg, 1998, 2005; Hillier, 2000; Hughes *et al*., 2001). Similarly, exposure of the developing follicle to inappropriately high concentrations of LH with the flare-up protocol in assisted reproduction may adversely affect the reproductive process, in the form of lower pregnancy rates and increased early pregnancy losses (Daya, 2001).

In summary, current concepts of gonadotrophic control of ovarian function and clinical evidence have established that both a 'threshold' and a 'ceiling' for LH concentrations (framing the so-called LH 'window') exist during the follicular phase of menstrual and induced cycles (Hillier, 1994, 2000; Balasch and Fábregues, 2002; Shoham, 2002; Balasch, 2004). Therefore, concentrations of LH should be neither too high nor too low during ovulation induction. During the second half of the follicular phase, as plasma FSH concentrations decline, the LH-dependent phase of pre-ovulatory follicular development

proceeds normally only if LH is present at concentrations over the threshold concentration and below the ceiling value. When the ceiling is exceeded at the midcycle surge of LH, further division of granulosa cells ceases as luteinization proceeds.

Clinical implications: the need for exogenous LH for ovulation induction and controlled ovarian hyperstimulation

On the basis of the above physiological evidence, now that pharmaceutically 'pure' rFSH and rLH are available, it is possible to develop improved clinical strategies for stimulating ovarian function. Those who stand to benefit are women receiving treatment for ovulatory dysfunction and those with normal ovarian function undergoing assisted reproductive techniques. The therapeutic aim in each group, however, is quite different. In the former, it is desirable to stimulate mono-ovulation with a view to conception occurring *in vivo*. In the latter, the aim is to stimulate multiple follicular development. The challenge is to tailor therapy with FSH and LH, alone or in combination, according to the outcome desired.

Induction of ovulation in WHO group II anovulatory patients

Ovulatory disturbances are present in about 15–25% of couples presenting for an infertility evaluation. Most infertile anovulatory patients fall into the WHO group II (normogonadotrophic anovulation) category, and the great majority of these women are diagnosed as having PCOS. These women are well oestrogenized and have normal FSH concentrations, but LH may be elevated (Hull, 1987; Hill, 1988; Speroff *et al*., 1994; American College of Obstetricians and Gynecologists, 2002; Balasch, 2004; Huirne *et al*., 2005). Elevated serum LH and disturbed intraovarian regulation of FSH action are endocrine features in PCOS (Taymor, 1996; Fauser and Van Heusden, 1997), and early studies both *in vitro* (Erickson *et al*., 1979) and *in vivo* (Seibel *et al*., 1984) provided evidence that the self-perpetuating state of biochemical imbalance so characteristic of PCOS could be interrupted in a physiological way when FSH is administered in a chronic low dose. Thus, although HMG and FSH preparations have both been used successfully for ovulation induction in PCOS (White *et al*., 1996), it is accepted that when endogenous LH is already elevated (for example, in PCOS), FSH alone is conceptually better (Hillier, 1994; Simoni and Nieschlag, 1995; Taymor, 1996; Balasch, 2004). In addition, it has been shown that LH concentrations significantly accumulate in the urine of PCOS patients receiving HMG for ovulation induction in a chronic low-dose protocol as compared with rFSH treatment (Balasch *et al*., 2003a). Finally, two reviews from The Cochrane Library on clinical trials investigating gonadotrophin therapy for ovulation induction in women with clomifene-resistant PCOS, concluded that no significant benefit could be demonstrated from urinary FSH versus HMG in terms of pregnancy rate, but a significant increase ovarian hyperstimulation syndrome (OHSS) associated with HMG was observed (Hughes *et al*., 1997; Nugent *et al*., 2002). According to experimental data, this could be explained by the reciprocal paracrine signalling between LH-stimulated theca cells and FSH-stimulated granulosa cells, which could bring about follicular hypersensitivity to FSH (Smyth *et al*., 1995). In contrast, a recent randomized, placebo-controlled, dose-finding study showed that, in WHO group II patients over-responding to FSH during ovulation induction, doses of rLH up to 30 μg/day are well tolerated in the late follicular phase and appear to increase the proportion of patients developing a single dominant follicle (Hugues *et al*., 2005). This study thus supports the 'LH ceiling' concept discussed above, whereby addition of a high dose of LH is able to control follicular growth by inducing atresia of developing follicles.

Induction of ovulation in WHO group I anovulatory patients

WHO group I anovulation or hypogonadotrophic hypogonadism (HH) is a much less frequent condition than PCOS, characterized by reduced hypothalamic or pituitary activity and resulting in abnormally low serum concentrations of FSH and LH and negligible oestrogen activity. The treatment of profoundly hypogonadotrophic women with urinary FSH or rFSH alone induces multiple follicle development but is associated with ovarian endocrine abnormalities and low oocyte fertilization rates (Balasch *et al*., 1995; Balasch, 2004). These findings, which are in agreement with the above-discussed current concepts on gonadotrophic control of folliculogenesis, indicate that, in spite of apparently normal follicular development induced by FSH, some exogenous LH is strictly necessary to optimize ovulation induction in terms of both drug requirements and clinical results. rLH thus appears to be an ideal adjunct therapy to rFSH in women with HH. Until recently, HMG was the only source of exogenous LH for this group of anovulatory women. The use of HMG containing fixed proportions of FSH and LH for ovulation induction in HH women has been linked to high prevalence of multiple folliculogenesis, which is considered as a major drawback to its use (Filicori *et al*., 1991; Martin and Hall, 1998).

At present, the use of rLH as a separate therapeutic agent allows the clinician to tailor the dose in order to stay below the 'LH ceiling' discussed above (Hillier, 1993, 1994; Balasch, 2004). In a pioneering multicentre dose-finding study (The European Recombinant Human LH Study Group, 1998) in which patients were randomized to receive rLH (0, 25, 75, or 225 IU/day) in addition to a fixed dose of rFSH (150 IU/day) it was concluded that rLH was found to: (i) promote dose-related increases in oestradiol and androstenedione secretion by rFSH-induced follicles; (ii) increase ovarian sensitivity to FSH, as demonstrated by the proportion of patients who developed follicles after the administration of a fixed dose of rFSH; (iii) enhance the ability of these follicles to luteinize when exposed to human chorionic gonadotrophin (HCG). In the study, it was shown that a daily dose of 75 IU rLH was effective in most women in promoting optimal follicular development, but a minority of patients may require up to 225 IU/day. Therefore, this pioneering study confirmed that there is individual variation in the dose of rLH required to promote optimal follicular development. Additionally, the early study (The European Recombinant Human LH Study Group, 1998) showed that increasing the dose of LH (up to 225 IU/day) during the follicular phase reduced the number of growing follicles, which might reflect a 'LH ceiling' effect as discussed above.

A more recent multicentre study (Burgués *et al*., 2001) confirmed that combined rFSH and rLH treatment induces follicular growth, ovulation, and pregnancy in a good proportion of hypogonadotrophic anovulatory patients, and is well tolerated. The doses of 150 IU rFSH and 75 IU rLH daily were found to be the most appropriate, but in a some patients doses above 75 IU rLH/day were necessary. Interestingly, this study clearly suggested that hypogonadotrophic patients having very low concentrations of endogenous LH (i.e. below the threshold for normal oestradiol biosynthesis and full follicular maturation) would necessitate higher doses of gonadotrophins, compared with women having adequate basal LH concentrations, to reach the criteria necessary for HCG administration (Burgués *et al*., 2001). In fact, there was individual variation in the dose of both LH and FSH necessary to induce ovulation depending on basal LH concentration, thus emphasizing the importance of administering FSH and LH separately, at least in some women.

Therefore, both studies (The European Recombinant Human LH Study Group, 1998; Burgués *et al*., 2001) confirmed that there is individual variation in the dose of LH (but also FSH) required to promote optimal (mono)folllicular development. Further refinement of the dosing schedule of both FSH and LH to minimize the likelihood of multiple ovulation occurring in these patients is now possible, with the availability of monotherapeutic recombinant gonadotrophic agents (Hillier, 1993, 2000). Thus, enhancing the LH environment would provide a means of inducing atresia in secondary follicles and promoting growth of a minimal number of pre-ovulatory follicles ('LH ceiling concept'). In fact, a recent study (Loumaye *et al*., 2003) on the subject involved patients with hypogonadotrophic hypogonadism who were treated with increasing doses (every 7 days) of rFSH (starting dose of 112.5 IU/day), according to patients' ovarian response, along with a fixed dose of 225 IU/day of rLH. When at least one follicle reached a diameter of 10–13 mm, the patients were randomized to three different groups: the first group continued treatment with both drugs; the second continued rLH and received a placebo substitute for rFSH; and the third continued rFSH and received a placebo substitute for rLH. When one follicle reached 18 mm in mean diameter, ovulation was triggered by the administration of 10,000 IU of HCG. The results of this study clearly demonstrated that the number of follicles >11 mm in diameter on the day of HCG injection was significantly lower in the rLH/placebo group in comparison with the rFSH/placebo group. This study performed in hypogonadotrophic hypogonadism patients, who are the best and only true models for investigating the physiology of gonadotrophin actions on the ovary, emphasizes the delicate balance and need for both FSH and LH in normal follicular development.

Finally, it is of note that a normal pregnancy was obtained after administration of high-dose rLH alone to support final stages of follicular maturation in a woman with long-standing hypogonadotrophic hypogonadism (Balasch and Fábregues, 2003). This supports the notion that once an appropriate (i.e. LH-responsive) stage of follicular development has been achieved in response to treatment with FSH, there are theoretical grounds for reducing or completely withdrawing FSH and maintaining tonic stimulation of the dominant follicle with exogenous LH (Hillier, 2000). Thus, it is possible that a dual advantage of high-dose rLH may exist in the form of promoting the terminal maturation of a single pre-ovulatory follicle, and simultaneously arresting the development of multiple less mature follicles that would otherwise occur in response to treatment with FSH.

Induction of multiple follicular development in assisted reproductive treatment cycles

Although the first IVF pregnancy occurred after induction of follicular growth with HMG (Steptoe and Edwards, 1976), the widespread application of this regimen was abandoned because stimulation with HMG and HCG shortened luteal phases in a manner directly proportional to the output of urinary oestrogens by the patients, sometimes to 7 or 8 days, severely restricting the chances of implantation (Fowler *et al*., 1978; Edwards *et al*., 1980). Although progestogens and oestradiol supplements were used for luteal support, they might have impaired corpus luteum activity since they immediately reduced levels of plasma progesterone. This probably explained the lack of pregnancies over some years, except for brief increases in HCG-β in a few patients, indicating short-lived (biochemical) pregnancies (Edwards *et al*., 1980; Steptoe *et al*., 1980; Edwards and Brody, 1995; Edwards, 2005).

At present, it is well established that successful IVF and embryo transfer require both stimulation of the ovary and suppression of the pituitary (Barbieri and Hornstein, 1999; Felberbaum *et al*., 2005). Thus, exogenous gonadotrophins and GnRH analogues are the key hormones required to maximize IVF success, with the long protocol of GnRH agonist being the most commonly adopted protocol for assisted reproductive treatment cycles worldwide. The low endogenous LH concentrations achieved with GnRH agonists in some cases may amplify the differences, if any, in treatment outcome seen with the use of HMG and FSH-only preparations. The recent availability of GnRH antagonists, which can cause more profound LH suppression than GnRH agonists, adds further interest to the subject.

Assisted reproduction treatment in general population

Several facts support the concept that LH administration is not needed in the vast majority of patients undergoing assisted reproductive treatment in cycles stimulated with rFSH in down-regulated women (long protocol of GnRH agonist) or in association with GnRH antagonist: (i) the switch in stimulation regimens using down-regulation with GnRH agonist to a more widespread use of FSH-only preparations, without LH supplementation, has been associated with an increased rate of overall programme success (Wikland, 1999; FIVNAT, 1999, 2000; Cramer *et al*., 2000); (ii) according to both case–control and cohort studies by us, LH serum measurements in the mid-follicular phase and even throughout the follicular phase during ovarian stimulation with rFSH cannot predict ovarian response and assisted reproductive treatment outcome in down-regulated women (Balasch *et al*., 2001a; Peñarrubia *et al*., 2003). Even in conditions of profound LH suppression, such as cycles treated with a depot GnRH and a fixed low gonadotrophin dose (both of which are neither standard practices nor absolutely first choice in assisted reproductive treatment), we found that supplemental LH may be required in terms of treatment duration and gonadotrophin consumption but, in spite of this, oocyte and

embryo yield and quality were significantly higher with the use of rFSH compared with HMG (Balasch *et al*., 2003b); (iii) rLH supplementation to rFSH does not improve ovarian stimulation and assisted reproductive treatment outcome in pituitary-suppressed women receiving the long protocol of GnRH agonist; even more, it may have a negative impact on oocyte maturation and/or implantation rates mainly in patients younger than 35 years (Balasch *et al*., 2001b; Marrs *et al*., 2004); (iv) in the early clinical trials comparing GnRH agonist and GnRH antagonist for assisted reproductive treatment, pregnancy rates in the GnRH antagonist groups were similar, irrespective of using rFSH or HMG for ovarian stimulation (Huirne and Lambalk, 2001). On the other hand, recent studies have shown that LH concentrations after GnRH antagonist administration do not influence pregnancy rates in IVF embryo transfer, and even more, profound LH suppression after GnRH antagonist administration is associated with a significantly higher ongoing pregnancy rate after IVF (Kolibianakis *et al*., 2004; Merviel *et al*., 2004). Finally, recent clinical trials have demonstrated that rLH supplementation to rFSH during GnRH-antagonist administration in assisted reproductive treatment cycles does not improve IVF outcome (Cédrin-Durnerin *et al*., 2004; Griesinger *et al*., 2005); (v) a very recent systematic review of the literature (Kolibianakis *et al*., 2006) concluded that the available evidence suggests that, among women with normal ovulation or WHO II oligo-anovulation, low endogenous LH concentrations during ovarian stimulation for IVF using GnRH analogues (agonist or antagonist) are not associated with a decreased probability of ongoing pregnancy beyond 12 weeks. On the contrary, this review concluded that there is evidence to suggest that the opposite may be true (Kolibianakis *et al*., 2006). In this respect it is worth noting that a study in profoundly down-regulated young oocyte donors showed that the inclusion of exogenous LH activity (in the form of 1 ampoule/day HMG from stimulation day 5) in the ovarian stimulation protocol with rFSH can have beneficial or detrimental effects on oocyte yield and quality, depending on the concentration of endogenous LH, thus supporting the concept of a 'window' for LH requirement in ovarian stimulation (Tesarik and Mendoza, 2002).

Therefore, according to the above evidence it seems clear that there is no need for administering exogenous LH for assisted reproductive treatment in the general population if daily doses of an appropriate GnRH agonist (in terms of the substance, formulation, and dosage) and the appropriate approach of rFSH administration are used. Notwithstanding this, a need for some LH supplementation may be evidenced in some women, depending on the extent to which the endogenous serum LH is suppressed by concomitant GnRH agonist therapy, the direct effect of the latter on the ovary, and the protocol of gonadotrophin administration used. Thus, recent randomized studies tested whether LH supplementation during controlled ovarian hyperstimulation, as opposed to increasing the daily rFSH dose, can improve the outcome in down-regulated normo-ovulatory normogonadotrophic patients who show an initial hyporesponsiveness to rFSH in the form of a steady response characterized by a normal follicular recruitment to age- and body mass index (BMI)-appropriate rFSH dosages on treatment days 5–7, but showing a plateau on follicular growth (no increase in the oestradiol concentration and in the follicular size) on days 8–10 of stimulation, in spite of continuing the same rFSH dosage (De Placido *et al*., 2001, 2004, 2005; Ferraretti *et al*., 2004). These women have to be distinguished from the typical poor responder in whom the detection of a few antral follicles during the early stages of stimulation is followed by later cancellation of the cycle due to insufficient follicular growth. From these studies (De Placido *et al*., 2001, 2004, 2005; Ferraretti *et al*., 2004) it was concluded that LH activity supplementation (in the form of HMG or rLH) is more effective than increasing the dose of rFSH in terms of ovarian outcome in patients showing a hyporesponsiveness to monotherapy with rFSH in the midfollicular phase of assisted reproductive treatment cycles. In addition, those studies demonstrated that the use of rLH is more effective than HMG in order to rescue the assisted reproductive treatment cycles, and the daily dose of 150 IU rLH seem to give better results than 75 IU in this regard (De Placido *et al*., 2004; Ferraretti *et al*., 2004).

Patients of advanced reproductive age and proposed individualized approaches

The two previous correspondents (Kol, 2005; Humaidan, 2006) suggest three additional subgroups of assisted reproductive treatment patients who would benefit from LH supplementation: down-regulated women of advanced reproductive age, those women having a drop in LH concentrations from day 1 to day 8 of stimulation in down-regulated cycles, and patients with high LH concentrations on stimulation day 8 after a long GnRH agonist down-regulation. This is not well supported by current evidence.

Two recent reports (Humaidan *et al*., 2004; Marrs *et al*., 2004) suggested that rLH supplementation from the mid- to late-follicular phase in women undergoing assisted reproduction with GnRH agonist down-regulation and stimulation with rFSH, may increase implantation rates in patients ≥35 years but not in younger women. One of these studies (Humaidan *et al*., 2004), however, was based on a low number of patients aged ≥35 years and, as stressed by the authors, their results require additional studies for confirmation. In the second study (Marrs *et al*., 2004), the clinical pregnancy rate was similar in women aged ≥35 years who received both rFSH and rLH and those stimulated with rFSH alone, but the difference in clinical pregnancy rates was significantly higher in favour of the rLH-supplemented patients when only the subgroup of women undergoing their first assisted reproductive treatment cycle were considered. This subgroup of patients, however, also had significantly more embryos transferred in the rFSH + rLH group. Predicted clinical pregnancy rates from a regression logistic model adjusted for the number of embryos transferred indicated no significant difference between rFSH + rLH and rFSH treatment groups, although the regression model also demonstrated that the higher number of embryos replaced in the LH-supplemented group did not explain the higher pregnancy rate (Marrs *et al*., 2004).

In an even more recent prospective randomized clinical trial including a total of 120 consecutive normogonadotrophic infertile women aged ≥35 years undergoing their first cycle of assisted reproductive treatment, it was concluded that rLH supplementation does not increase ovarian response and implantation rates in patients of older reproductive age stimulated with rFSH under pituitary suppression (Fábregues *et al*., 2006). Interestingly, a 'ceiling effect' was evidenced in

this study where rLH-supplemented patients showed impaired follicular development and oocyte yield compared with patients receiving rFSH alone (Fábregues *et al*., 2006). Considering some methodological differences between the three studies (Humaidan *et al*., 2004; Marrs *et al*., 2004; Fábregues *et al*., 2006) mainly regarding the sample size, patients' selection criteria, the type of GnRH agonist used, the stimulation day when LH supplementation was started, and even the rFSH/rLH ratios used, further studies on the subject are warranted before a definite indication for routine rLH supplementation in women of reproductive age undergoing assisted reproductive treatment is established. Thus, it has been reported that supplementation with rLH 75 IU/day in assisted reproductive treatment patients aged over 38 years stimulated with rFSH under pituitary down-regulation, may improve early follicular recruitment and the number of metaphase II oocytes obtained (Gómez-Palomares *et al*., 2005). However, no beneficial effect in the rLH-treated group was observed in that study in terms of embryo yield and pregnancy rates (Gómez-Palomares *et al*., 2005).

We (Balasch *et al*., 2003b; Peñarrubia *et al*., 2003; Fábregues *et al*., 2005) and others (Cabrera *et al*., 2005) have reported on LH measurements throughout the follicular phase in assisted reproductive treatment cycles stimulated with rFSH alone under pituitary suppression with the long protocol of GnRH agonist. In these studies the suppressed levels of early-, mid- and late-follicular serum LH were not predictive of ovarian response or pregnancy. This was confirmed using receiver operator characteristic (ROC) analysis and the area under the curve (AUC, which corresponds to an integrated hormone concentration for the chosen days). Thus, considering these facts, we must agree with Dr Humaidan (2006) when stressing that the use of changes in LH serum concentration from day 1 to day 8 does not seem to be a useful marker to identify those patients needing supplementation with exogenous LH. In this regard, we would stress the following. It is known that in a suppressed pituitary gland the dose needed to maintain suppression gradually decreases with the length of treatment (Sandow and Donnez, 1990). On the other hand, as ovarian stimulation with gonadotrophins progresses, the suppression of pituitary gonadotrophin secretion becomes more effective and the concentrations of endogenous LH decrease (Esposito *et al*., 2001; Peñarrubia *et al*., 2003). Thus, a nadir in LH serum concentrations is usually obtained in the mid-follicular phase and recent studies stressed the potential adverse effects of suppression of LH during stimulation days 7–8 in normogonadotrophic women undergoing assisted reproductive treatment (Fleming *et al*., 2000; Westergaard *et al*., 2000; Humaidan *et al*., 2002).

Interestingly, halving the daily dose of GnRH agonist once ovarian suppression is evidenced is current practice in different assisted reproduction programmes, such as ours, using the GnRH agonist long protocol. This may be theoretically important in two respects. First, it might contribute to avoiding a too profound suppression of concentrations of LH during follicular development; second, it could reduce a potentially detrimental direct effect of GnRH agonist on ovarian steroidogenesis (Balasch and Howles, 1999). In this respect it is important to note that an increase in serum LH concentrations from the mid-follicular phase to the day of HCG administration is observed when the daily dose of GnRH agonist is halved (Peñarrubia *et al*., 2003), but not when profound pituitary suppression is obtained with the use of depot GnRH agonist preparations (Filicori *et al*., 1999, 2001; Balasch *et al*., 2003b). On the other hand, it has recently been reported (Fábregues *et al*., 2005) that halving the standard daily dose of triptorelin at the start of ovarian stimulation in down-regulated women stimulated with rFSH is enough for pituitary suppression and was associated with significantly higher LH serum concentrations in the follicular phase. Although this did not translate into higher serum concentrations of androstenedione and oestradiol and had no significant effect on ovarian response and the outcome of assisted reproduction treatment, it means that further increase over a 'threshold level' of LH action needed to gain a response will not induce a greater increase in ovarian stimulation, and may even be detrimental if, as discussed above, the 'ceiling effect' is reached. Therefore, the use of rLH supplementation in down-regulated patients having high LH serum concentrations on stimulation day 8 as proposed by Humaidan (2006) on the basis of the analysis of small groups of patients (Humaidan *et al*., 2004), seems clearly questionable and lacking in proven scientific rationale.

The daily dose of LH to induce a ceiling effect in assisted reproduction technology cycles remains to be established and it is not an easy task, mainly considering that: (i) there is growing evidence showing that circulating LH measurements do not accurately reflect LH administration (The European Recombinant Human LH Study Group, 1998; Burgués and The Spanish Collaborative Group on Female Hypogonadotrophic Hypogonadism, 2001; De Placido *et al*., 2001, 2005; Ferrareti *et al*., 2004; Fábregues *et al*., 2006); (ii) serum LH concentrations assayed by immunoassay do not necessarily reflect circulating LH bioactivity (Jaakkola *et al*., 1990; Schroor *et al*., 1999); (iii) the regime and the dose of GnRH agonist in connection with ovarian stimulation have an important effect on the residual LH activity in circulation (Humaidan *et al*., 2002; Tesarik and Mendoza, 2002). In fact, the GnRH agonist seems to be the major effect modifier of endogenous LH concentrations, depending on the substance, formulation, and dosage used (Westergaard *et al*., 2001; Balasch, 2004), and it has been shown that the currently used dosages of GnRH agonists in assisted reproduction technology are too high, resulting in unphysiologically low LH concentrations (Janssens *et al*., 2000). The importance of GnRH agonist administration in assisted reproduction cycles is further supported by studies showing that an early discontinuation of the agonist during a short-term or a long-term protocol may decrease LH serum concentrations ever further, and/or be detrimental to follicular development and steroid synthesis (Fujii *et al*., 1997; Cédrin-Durnerin *et al*., 2000). Thus, using daily doses of an appropriate GnRH agonist (leuprolide or triptorelin having lower potency than buserelin) but not depot preparations (having a more profound suppressive effect on the pituitary and ovaries than daily doses) and an appropriate regimen of rFSH administration, there is no need for additional exogenous LH supplementation in the vast majority of women undergoing assisted reproduction treatment (Balasch and Fábregues, 2002; Balasch, 2004).

Conclusions

LH play an essential physiological role in follicle steroidogenesis and development and oocyte maturation. Thus, exogenous LH is an essential tool in ovulation induction. Current concepts

of gonadotrophic control of ovarian function have established that both a 'threshold' and a 'ceiling' for LH concentrations exist during the follicular phase of menstrual and induced cycles. Therefore, concentrations of LH should be neither too high nor too low during ovulation induction, in order to not compromise reproductive performance. This implies that: (i) it is inappropriate to use LH-containing products for ovarian stimulation in PCOS patients; (ii) treatment with exogenous LH is strictly necessary in WHO group I anovulatory patients; and (iii) resting concentrations of serum LH are sufficient in the vast majority of women receiving ovarian stimulation with FSH-only products in association with GnRH analogues (agonists or antagonists) for assisted reproduction treatment. Although there may be specific subgroups of endocrinologically healthy assisted reproduction treatment patients needing LH supplementation, it is well accepted that the best and only true model to investigate any LH hypothesis correctly is the hypogonadotrophic woman who may be totally LH deficient. It seems clear that normally ovulating women with pituitary down-regulation are not comparable to WHO group I anovulatory patients, as in most cases an absolute deficiency does not really exist, as demonstrated by a very different steroidogenic response to FSH alone (Hillier, 1994, 2000). In addition, the use of GnRH analogues represents a major effect modifier. Therefore, to establish the threshold value of LH that should be used to discriminate between LH concentrations considered sufficient and those considered too low or too high in assisted reproduction treatment patients is not an easy task. Thus, more data are necessary before evidence-based recommendations regarding exogenous LH supplementation in ovarian stimulation protocols for assisted reproduction treatment can be provided.

On the other hand, it should be noted that, for years, HMG (containing FSH- and LH-like activity in the form of HCG) has been the only gonadotrophin available for clinical use, and most current available data on ovulation induction come from clinical experience with this urinary preparation. Recombinant gonadotrophins (FSH, LH, HCG) are now available and they have higher biopotency and bioactivity than their urinary counterparts. Therefore, results with HMG in different clinical conditions cannot be fully extrapolated to recombinant drugs, and thus further refinement in terms of recombinant gonadotrophins' requirements may be necessary to optimize ovulation-induction protocols. The recent availability of rLH, the only source of pure, high-quality, stand-alone physiological LH for therapeutic purposes, opens new perspectives on the subject. Thus, by dissociating LH administration from FSH, it is possible to improve safety by promoting mono-ovulation. This is further stressed by the possibility of using some 'antifolliculogenic' effects of high concentrations of rLH, which may promote mono-ovulation while inducing atresia of secondary follicles. Also, recent preliminary studies showed that it is possible to approximate the situation in a natural ovarian cycle encouraging the selective maturation of a single LH-responsive pre-ovulatory follicle and minimizing the likelihood of multiple ovulation. Once the appropriate stage of follicular development (i.e., LH-responsive) has been achieved in response to treatment with rFSH, this hormone is reduced or completely withdrawn and tonic stimulation of the dominant follicle is maintained with rLH. However, it remains to be established which dose and frequency of LH administration are adequate to sustain the development of a single pre-ovulatory follicle without exceeding the ceiling beyond which LH induces premature luteinization or disordered oocyte development.

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Commentary

To add or not to add LH: comments on recent commentaries

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Abstract

Human reproductive evolution, involving a complex interaction of the hypothalamic–pituitary–ovarian axis, the release of pulsatile and threshold concentrations of gonadotrophins and positive and negative feedback systems, has ensured the release of a single viable egg and functioning corpora lutea in the natural menstrual cycle. The use of follicular stimulation regimens to obtain multiple eggs has resulted in a compromise – in terms of the risk of ovarian hyperstimulation, cost, multiple pregnancies, wastage of or the need for cryopreservation of surplus embryos. Even some women with apparently normal menstrual cycles might become 'poor responders' when administered with follicular stimulants, and we still do not know if the incidence of oocyte aneuploidy is artificially raised after stimulation. After the advent of recombinant FSH and LH, the precise roles of these hormones individually needs to be elucidated to understand the physiological requirements for successful ovarian stimulation in each woman undergoing IVF, to maximize her chance and minimise attendant risks. One of the key debates is the role of LH, which in the natural cycle is significant, but may be redundant during ovarian stimulation for IVF. Current outcome indicators are crude when attempting to understand the physiology, and more basic research and randomized, focused clinical trials are needed.

Keywords: *follicular stimulation, IVF, recombinant gonadotrophins, recombinant LH*

I read with interest the recent commentaries by Dr Kol (Kol, 2005) and Dr Humaidan (Humaidan, 2006). Our group as been looking at the problem of exogenous recombinant LH (rLH) on rFSH stimulation after a long gonadotrophin-releasing hormone (GnRH) agonist down-regulation protocol, since the very inception of this concept. In a preliminary study (Lisi *et al*., 2001) 12 patients (17 cycles) who needed >3000 IU of rFSH on previous follicular stimulation attempts for IVF using rFSH, underwent further attempts (a total of 12 cycles) using rFSH supplemented with rLH from day 7 of stimulation: there was a significant increase in the incidence of fertilization (60.9 versus 86.0%; $P = 0.006$) and clinical (ongoing) pregnancies (1 versus 6; $P = 0.022$). The higher incidence of fertilization increased the mean number of embryos transferred per patient, although in this small group the data were not significant (1.75 versus 2.71). From these results we deduced that exogenous supplementation with rLH might be beneficial for patients down-regulated and undergoing assisted reproduction techniques, although the mechanisms remained unclear. In a further study (Lisi *et al*., 2003) we investigated the effect of supplemental rLH upon the stimulation and outcome profiles of a group of 41 women who in a previous treatment cycle required excessive rFSH (>2500 IU) to reach follicular maturation. No difference was observed in fertilization or the mean number of embryos available for transfer. There was a significant ($P < 0.004$) increase in the number of grade 1 embryos in these women, clinical pregnancy rate ($P = 0.05$) and the rate of implantation ($P < 0.05$), and again it was concluded that patients who require excessive (>2500 IU) rFSH for follicular stimulation after down-regulation might benefit from superimposing rLH, which, in these studies, improved embryo grading, implantation and clinical pregnancy rates. In a larger study (Lisi *et al*., 2002) we evaluated the use of rLH supplementation in an unselected group of IVF patients undergoing follicular stimulation with rFSH after pituitary down-regulation. Group A comprised 122 cycles administered rFSH and rLH, while Group B included 331 cycles using rFSH only during the same period of treatment. There was no significant difference in any of the endocrine, embryological