International Journal of Women's Health

Dovepress
open access to scientific and medical research

Open Access Full Text Article

REVIEW

Profile of follitropin alpha/lutropin alpha combination for the stimulation of follicular development in women with severe luteinizing hormone and follicle-stimulating hormone deficiency

This article was published in the following Dove Press journal:
International Journal of Women's Health
25 May 2016
Number of times this article has been viewed

Leonardo Rinaldi
Helmy Selman

One Day Medical Center, IVF Unit, Rome, Italy

Abstract: A severe gonadotropin deficiency together with chronic estradiol deficiency leading to amenorrhea characterizes patients suffering from hypogonadotropic hypogonadism. Administration of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) to these patients has been shown to be essential in achieving successful stimulation of follicular development, ovulation, and rescue of fertility. In recent years, the availability of both recombinant FSH (rFSH) and recombinant LH (rLH) has provided a new therapeutic option for the stimulation of follicular growth in hypopituitary–hypogonadotropic women (World Health Organization Group I). In this article, we review the data reported in the literature to highlight the role and the efficacy of using recombinant gonadotropins, rFSH and rLH, in the treatment of women with severe LH/FSH deficiency. Although the studies on this issue are limited and the experiences available in the literature are few due to the small number of such patients, it is clearly evident that the recombinant gonadotropins rFSH and rLH are efficient in treating patients affected by hypogonadotropic hypogonadism. The results observed in the studies reported in this review suggest that recombinant gonadotropins are able to induce proper follicular growth, oocyte maturation, and eventually pregnancy in this group of women. Moreover, the clinical use of recombinant gonadotropins in this type of patients has given more insight into some endocrinological aspects of ovarian function that have not yet been fully understood.

Keywords: follicular growth, gonadotropins, implantation, ovarian stimulation, pregnancy

Introduction

A severe gonadotropin deficiency together with chronic estradiol deficiency leading to amenorrhea characterizes hypogonadotropic hypogonadism (HH). Deficiency in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels may result from either hypothalamic or pituitary causes.

HH is most frequently acquired and caused by a number of hypothalamic or pituitary pathological processes. When secondary causes of HH can apparently be excluded, the diagnosis of idiopathic or isolated HH factors may be recognized. Exogenous administration of both FSH and LH in women with HH has been shown to be essential in achieving successful stimulation of follicular development, ovulation, and fertility restoration.[1–3] In women with HH and normal pituitary function, pulsatile

Correspondence: Leonardo Rinaldi
One Day Medical Center, IVF Unit,
Via Attilio Ambrosini 114/126, 00147,
Rome, Italy
Tel/fax +39 06 4521 2038
Email leo.rinaldi@tiscali.it

submit your manuscript | www.dovepress.com
Dovepress
http://dx.doi.org/10.2147/IJWH.S88904

gonadotropin-releasing hormone (GnRH) therapy can be used to induce the periodic release of FSH and LH, leading to proper ovulation.[4–8] The use of a portable pump injecting GnRH either intravenously or subcutaneously for several weeks can cause practical and clinical problems. Additionally, the use of daily injections of exogenous gonadotropins has proven to be as effective as GnRH therapy in inducing ovulation in all HH women, including those with HH of pituitary origin, when GnRH therapy is clearly not effective. For many years, human menopausal gonadotropin, which provides both FSH and LH activities, has been applied as the drug of choice in HH patients needing restoration of ovulation.[3,5,7,9] In recent years, the availability of both recombinant FSH (rFSH) and recombinant LH (rLH) has provided a new therapeutic option for HH patients. In 1997, Agrawal et al[10] reported the first birth in a hypopituitary–hypogonadotropic woman (World Health Organization [WHO] type I) following stimulation of follicular growth with rFSH and rLH.

The aim of this article is to review the data reported in the literature on the efficacy of the administration of both rFSH and rLH in the treatment of women with severe LH/FSH deficiency.

Role of gonadotropins in follicular growth and ovulation

The two cell–two gonadotropin theory

The two cell–two gonadotropin theory was established to understand the roles of LH and FSH, as well as their correlation with the physiological hormonal milieu, which lead to follicular growth, maturation, and ovulation in the woman. In the antral follicle, LH receptors are present only in the theca cells, whereas granulosa cells express only FSH receptors. Androgen production and release during folliculogenesis is dependent on the stimulation of the theca cells by LH. During follicular growth, the theca cells produce androgens in response to LH, which are then converted into estrogen by FSH-induced aromatase in the neighboring granulosa cells in the selected growing follicles. In the midfollicular phase, LH receptors begin to appear on the granulosa cells and are involved in the induction and maintenance of a complex paracrine system (which involves inhibin B and insulin-like growth factor [IGF-1]) necessary for granulosa cell growth and regulation of oocyte maturation. According to the so-called "spare receptor hypothesis",[11] at a given time, when inhibin B and IGF-1 are adequately secreted, androgen synthesis and release are optimal even with $<1\%$ of LH receptors occupied. The decline in FSH level, which occurs during follicular growth, has a key role in the selection of the dominant follicle, as evinced by the fact that on the selected follicle, FSH receptors appear with a lower threshold than on the other growing follicles. On the other hand, according to the LH ceiling theory, each follicle would have an upper limit of stimulation, which is higher in larger follicles and lower in smaller ones. LH would promote leading follicle progression when its concentration is less than its ceiling, and it would cause the degeneration of secondary follicles by overcoming their ceiling.[12] At midcycle, the LH surge triggers the final oocyte maturation and ovulation and, at the same time, prevents further growth of granulosa cells, leading to absence of atresia of dominant follicles.[13]

Clinical and endocrinological features of HH

HH may result either from absent or inadequate hypothalamic GnRH secretion or from failure of pituitary gonadotropin secretion. Several congenital and acquired causes, including functional and organic forms, have been associated with this condition.

Congenital isolated forms of HH are characterized by partial or complete lack of pubertal development due to a deficiency in GnRH-induced gonadotropin secretion, in the absence of anatomical abnormalities in the hypothalamic and pituitary region, although baseline and reserve testing of the remaining pituitary hormones are normal. This could also be associated with olfactory dysfunction, as demonstrated in ~50%–60% of patients (Kallmann syndrome).[14] From a genetic point of view, congenital isolated forms of HH constitute a very heterogeneous condition, and many genes are implicated in the mechanism of HH.[14–16]

Acquired causes of HH are mainly invasive disorders of the hypothalamic–pituitary tract, such as pituitary adenomas sarcoidosis, craniopharyngiomas, histiocytosis, and other central nervous system tumors. In these cases, associated pituitary hormone deficiencies are the common result.[16] Adult onset of isolated pituitary gonadotropin deficiency can be related to systemic disorders, drugs (glucocorticoid, opiates, and psychotropic agents), functional abnormalities due to excessive exercise, hyperprolactinemia, nutritional deficits, psychological distress, or even idiopathic condition.[17]

Actually, the discussion of the various causes of hypogonadism is not inherent to our article as the clinical presentations of the congenital forms are still variable and debatable.

From a clinical point of view, amenorrhea, infertility, decreased libido, and osteoporosis are the main symptoms caused by anovulation and chronic estradiol deficiency,

which in turn are related to the severe gonadotropin deficiency determined by HH.[15]

Follitropin alpha and lutropin alpha

FSH and LH are glycoproteins composed of two noncovalently linked protein subunits, the alpha and beta subunits.[18,19] The alpha subunit contains 92 amino acids and is identical in FSH, LH, and human chorionic gonadotropin (hCG), whereas the beta subunit varies between the different glycoproteins and confers unique receptor specificity and specific biological properties.[20] Its biological activity is provided by the attachment of carbohydrate moieties, forming heterodimers; the extent and pattern of glycosylation convey the spectrum of different charges, bioactivities, and half-lives for each glycoprotein.[21,22] Endogenous gonadotropins exist in a number of different isoforms, which have similar amino acid sequences but differ in their terminal sialic acid content. Although gonadotropin isoforms influence a variety of biological activities, including cellular growth and development, steroidogenesis, and protein synthesis, their clinical roles are still to be determined.[23–27]

Recently, the use of genetic engineering and recombinant DNA technology has led to the development of the recombinant human gonadotropin preparations follitropin alpha and follitropin beta.[28,29] Follitropin alpha was the first recombinant human FSH (hFSH) preparation successfully implemented in ovarian stimulation protocols. Its purity and in vivo bioactivity confer safety, efficiency, and tolerability advantages. More recently, lutropin alpha, the first rLH has also been produced and became commercially available for clinical use.[30,31]

Recombinant DNA technology used to produce follitropin alpha and lutropin alpha implements the incorporation of the gene encoded for the bioformation of each hormone into a genetically engineered Chinese hamster cell line. Subsequently, the extraction and purification of rFSH and rLH are carried out by the use of immunochromatographic techniques.[28,32]

Follitropin alpha and lutropin alpha are glycoproteins structurally similar to endogenous FSH and LH. They contain similar alpha subunit and different beta subunits with specific bioactivities.[25,33] A different rFSH, follitropin beta, has been later produced and has become commercially available for clinical use.[34,35] Follitropin alpha resembles the natural FSH isoform detected at midcycle, whereas follitropin beta is similar to that detected in the early follicular phase.[36]

rFSH preparations have been introduced successfully in the treatment of couples with infertility problems. Some clinical trials have shown that rFSH is highly effective in terms of oocyte yield, embryo quality, and dose of FSH needed, with less risk of causing ovarian hyperstimulation syndrome (OHSS).[37,38] Other studies, however, have demonstrated that the efficacy of rFSH in terms of oocyte and embryo quality is not superior to that of urinary hFSH. Some authors have argued that the difference between the two types of FSH may be due to the presence of LH activity in hFSH preparations, which has a positive effect on oocyte maturation and embryo quality,[39,40] while other investigators have postulated that such differences may reside in the nature of FSH isoform activities.[39,41] FSH isoforms differ in their ability to bind to the target cell receptors surviving in the circulation and to induce biological responses in vivo and in vitro.[42,43] A significant difference exists between human-derived FSH and rFSH in terms of their glycosylation patterns and sialic acid content: hFSH contains a higher proportion of acidic isoforms, whereas rFSH contains a higher proportion of less acidic isoforms.[27,44–46]

This difference in the glycosylation pattern of FSH is reflected in its bioactivity, its clearance rate, and its biological function.[47–49] It has been suggested that the less acidic isoforms have a faster circulatory clearance and, thus, a shorter circulatory half-life[48] than the acidic isoforms.[50,51] However, a more recent study has shown that the slow clearance of the acidic isoform results in better follicular maturation and estradiol secretion compared with the less acidic isoform.[41] A growing body of evidence shows that follicular development patterns and oocyte quality are strongly affected by the FSH glycoform range, and that the requirements of the growing follicle may change during its progress through different stages of follicular development.[52,53] Indeed, the glycosylation patterns of the two types of FSH implemented for ovarian stimulation have an important role in oocyte maturation competence and clinical outcomes.[54]

On the other hand, human menopausal gonadotropin (hMG), widely used in a variety of infertility treatment protocols, has both FSH and LH, and sometimes hCG, activities. It contains 75 IU of FSH and 75 IU of LH, as measured by standard bioassays. Recently, more purified forms of hMG with specific activity of >2,500 IU mg for both FSH and LH activities are produced and they also contain some hCG activity. A highly purified form of hMG (hMG-HP) has become available, which contains more hCG (10 IU) and less LH (5 IU) than the other forms of hMG preparations, and nowadays, it is successfully used in ovarian stimulation regimens.

Currently, the rLH lutropin alpha has become commercially available,[32] and it has been successfully used

in combination with rFSH for infertility treatment, as an alternative to hMG, and the results achieved are as expected. Although the role of LH in sensitizing antral follicles to FSH still remains to be elucidated, it has been argued that LH is required for normal hormone production and normal oocyte and embryo development. Nevertheless, follicular responses to LH may depend upon the stage of follicular development, it is clearly evident that LH has an important role in the final oocyte maturation and ovulation trigger. Additionally, a new combination of rFSH and rLH (follitropin alpha + lutropin alpha) at a 2:1 ratio was first introduced for infertility treatment in 2007. Among the advantages of this combination, used for women requiring LH supplementation, is that both FSH and LH can be administered in a single injection, rather than two. However, it has been shown that a similar bioequivalence of rFSH and rLH was observed when they were administered either alone or in combination.[19,55,56]

Stimulation of follicular development with rFSH and rLH in women with profound FSH and LH deficiency: clinical results

Because both recombinant gonadotropins (rFSH and rLH) have become available, they have also been applied in the treatment of WHO type I anovulatory patients, ie, patients with oligomenorrhea/amenorrhea caused by HH. Due to the rarity of this condition (~1% of infertile patients), most of the initial studies described some isolated case reports on the administration of both rFSH and rLH to treat HH patients.[10,57–62]

In 1998, the European Recombinant Human LH Study Group[63] published the first study on the use of recombinant gonadotropins in a large group of HH women with the aim to determine the minimal effective dose of rLH for supporting rFSH-induced follicular development in LH- and FSH-deficient anovulatory women (HH). Thirty-eight infertile women with HH (baseline serum LH: ≤1.2 IU/L; mean ± SD: 1.0±0.1 IU/L; mean serum FSH: 1.6±1 IU/L) were enrolled in the study. Patients were randomly assigned to receive daily 0 IU, 25 IU, 75 IU, or 225 IU rLH, in addition to 150 IU rFSH daily, administered for up to 20 days. As expected, patients receiving rFSH without rLH supplement did not show successful follicular growth, whereas patients receiving rLH supplement exhibited improved ovarian response, as measured by the presence of at least one follicle >17 mm, a level of estradiol ≥400 pmol/L, and midluteal phase progesterone ≥4 nmol/L. The study also showed that the efficacy of stimulation increased with increasing dose of rLH administered. The authors suggested that the presence of a "ceiling effect" is related to the rLH dose: the group of patients who received 225 IU/d of rLH had a smaller number of growing follicles than the group who received 75 IU of rLH/d. This could reflect an LH ceiling effect, whereby some secondary follicles underwent atresia due to their high sensitivity to LH. Pregnancy rates (PRs) achieved were comparable to those in a previous study, wherein hMG had been administered to induce ovulation and pregnancy in HH women, ranging from a PR of 29% per cycle with a 75 IU rLH dose to a 20% PR per cycle with a dose of 225 IU of rLH.[63]

These data were confirmed by Loumaye et al,[64] studying 24 WHO type I patients and 36 WHO type II patients; they showed that rLH alone can trigger arrest of follicular growth in a significant number of patients, suggesting the existence of an "LH ceiling" during late follicular maturation.

Initially, Burgués et al[65] conducted another study on a large group of 38 WHO type I women, who were stimulated with an initial fixed dose of 150 IU of rFSH and 75 IU of rLH. The dose of rLH was adjusted where necessary. Eighty-four cycles underwent ovarian stimulation, of which 79 (94%) achieved sufficient follicular growth. Clinical pregnancies were established in 15 (39.5%) out of 38 patients, with a PR of 18% per started cycle and a PR of 22.4% per given hCG.

Six years later, Kaufmann et al[66] evaluated the efficacy of rFSH and rLH in HH women in a wide prospective, randomized, placebo-controlled, double-blind, multicenter study, involving 23 centers in three countries. The stimulation doses were flexible, with a starting dose of 75 IU of rLH and 150 IU of rFSH daily in all the patients. In the case of patients with suboptimal response, the rFSH dose was increased to a maximum of 225 IU, or decreased if necessary. In 27 out of 31 patients, follicular development was achieved in a maximum of three cycles, and 20 (74.1%) of those patients became pregnant. In a similar double-blind, randomized, placebo-controlled trial conducted in 25 medical centers in four countries, Shoham et al[67] investigated the safety and efficacy of administration of 75 IU of lutropin alpha in combination with follitropin alpha for follicular development induction in women with profound gonadotropin deficiency. They administered a fixed dose of 75 IU rLH and 150 IU rFSH to 27 HH patients and a fixed dose of 150 IU of rFSH and a placebo without rLH to 12 HH patients. They observed a significant improvement of follicular growth in patients treated with lutropin alpha compared to those patients in the placebo group (P=0.023): 66.7% (16 of 24) vs 20.0% (two out

of ten). Two patients of the study group had a positive hCG with a PR of 15% per given hCG and 8% per started cycle. The response rate of combined lutropin alpha and follitropin alpha treatment was similar to that (66.7%) reported by the European study[63] that used the same entry criteria.

Further study was conducted by O'Dea et al,[68] in order to evaluate the need for LH supplementation in women with gonadotropin deficiency and to assess the requirement of rLH supplementation to support rFSH for ovulation induction in anovulatory, amenorrhoeic women. A group of 43 patients underwent a total of 61 treatment cycles with rLH and rFSH. The patients were randomly assigned to receive 0 IU/d, 25 IU/d, 75 IU/d, or 225 IU/d of rLH and a fixed dose of 150 IU/d of rFSH. Only 15 out of 43 patients had a typical HH with LH level ≤1.2 IU/L and really needed rLH supplementation to achieve adequate follicular growth and maturation, while in the remaining amenorrhoeic women, with higher basal level of LH, proper ovulation was obtained by the administration of rFSH alone.

More recently, Carone et al[69] were the first to compare the efficacy of recombinant vs urinary gonadotropins in women with severe HH. Seventeen HH women were administered recombinant gonadotropins (150 IU rFSH + 75 IU rLH daily) for 27 cycles of stimulation, and 18 HH women received urinary gonadotropin (150 IU hMG-HP daily, which is equal to 150 IU FSH + 150 IU LH-like activity) for 43 cycles of stimulation. Their results showed that rLH is highly superior compared to hCG in supporting FSH-induced follicular development in WHO type I women in terms of PR: 55% per cycle in rFSH/rLH patients vs 23.2% per cycle in hMG-HP patients ($P<0.05$). Interestingly, no statistical differences were observed between hMG-HP and rFSH + rLH patients when considering only ovulation induction. Ovulation was achieved in 88% of hMG-HP cycles compared to 70% of recombinant cycles, suggesting that rFSH/rLH is equally efficient as hMG-HP in inducing ovulation. According to the authors, the difference in terms of pregnancies among the two groups of patients may be due to the different sources of LH activity present in the two pharmaceutical preparations. In fact, in hMG-HP, the LH activity is derived from urinary hCG.[70] Despite the fact that LH and hCG have 90% amino acid homology, an evident difference exists between them: hCG is characterized by the presence of a carboxyl terminal peptide and a high glycosylation pattern, whereas LH contains three *N*-linked residues. Although they act on the same receptor, they stimulate different bioactivity mechanisms.[71] Moreover, hCG has a longer half-life and capability of accumulation, which may contribute to LH receptors' desensitization and downregulation, when compared to LH.[72–76] Additionally, the differences between the two molecules may reside in their different interactions with the same receptor, and this could partially explain the significant differences in the clinical outcome.[69]

In a recent study, Papaleo et al[77] have analyzed the study reported by Carone et al[69] from a pharmacoeconomic point of view in order to develop a cost-effectiveness model, comparing between rFSH + rLH and hMG-HP. Their study indicates that the average cost per pregnancy is lower for patients treated with rFSH + rLH than for those treated with hMG-HP; this may be due to the strong impact of the efficacy of the recombinant gonadotropin therapy with respect to the urinary gonadotropin therapy. They found that rFSH + rLH is associated with a higher total cost (€3,453.50) and higher efficacy (0.87) compared with hMG-HP (€2,719.70) and lower efficacy (0.50), but the average cost estimated per pregnancy is around €3,990.00 for the recombinant strategy and €5,439.80 for the urinary strategy. The authors concluded that despite the higher acquisition cost in comparison to hMG-HP, using rFSH + rLH resulted in a higher pregnancy rate, which makes it the recommended choice of treatment when considering the cost-effectiveness of rLH used in supporting FSH-induced follicular growth in HH women.

Additionally, Awwad et al[78] investigated whether split daily doses of recombinant human LH is more effective than the single daily dose in supporting follicular development and ovulation in primary HH. In their study, 27 women with HH received a 150 IU fixed dose of recombinant hFSH daily, subcutaneously administered, supplemented with 75 IU dose daily of rLH. The patients received the therapy either as a single dose (n=9; single-dose group) or four equally divided doses (n=18; split-dose group). Although no statistical significance was observed between the two groups, the proportion of women in the rLH split-dose group who achieved proper ovulation (at least one follicle ≥17 mm in diameter, preovulatory serum estradiol ≥400 pmol/L, and a midluteal progesterone ≥25 nmol/L) was higher than in the single-dose group (72.2% vs 55.6%). Although no data were reported on pregnancy outcomes, the authors concluded that administering rLH in split daily doses appears to be superior in terms of ovulation induction compared with the traditional single daily dose.

The availability of pure recombinant human gonadotropin preparations (rFSH and rLH) helped to achieve more insight to confirm the two cell theory of ovarian steroidogenesis, which predicts that both FSH and LH are required to ensure adequate follicular growth and maturation. FSH alone is insufficient

Table 1 Use of rFSH and rLH to restore fertility in HH women: ovulation and pregnancy in relation to the rLH doses

Dose of rLH, IU	0	25	75/225
Number of patients with adequate ovulation induction, n/N	0/11	6/12	16/26
Number of pregnant patients, n	0	0	6

Note: Data from The European Recombinant Human LH Study Group[63] and O'Dea et al.[68]

Abbreviations: HH, hypogonadotropic hypogonadism; rFSH, recombinant follicle-stimulating hormone; rLH, recombinant luteinizing hormone.

for full follicular maturation and oocyte competence in the case of severe gonadotropin deficiency; nevertheless, using FSH alone has been demonstrated to be sufficient for ovarian stimulation in normogonadotropic women.[62,63,79]

Concerns about the minimal effective dose of rLH needed to induce appropriate ovulation in association with rFSH have been investigated only in two clinical trials.[63,68] These studies have tested the capability of different doses of rLH (0 IU, 25 IU, 75 IU, and 225 IU daily) to stimulate adequate follicular growth and maturation. As shown in Table 1, no follicular growth was seen in HH women when no rLH was administered, and this seems to be in accordance with the "two cell theory". The minimal dose of rLH necessary to achieve ovulation induction seems to be 25 IU, in association with a fixed dose of rFSH of 150 IU/d; nevertheless, no pregnancy was achieved in this group of patients. On the other hand, the proportion of patients achieving appropriate ovulation increased with increasing doses of rLH to 75 IU and 225 IU, and six pregnancies were achieved out of 26 treated patients, with a PR of 23%. This could be the consequence of a better hormonal milieu in these groups of patients.[63,68] Interestingly, an improvement in clinical results could be obtained when both rLH and rFSH doses were tailored to the patient's response to stimulation, and adjusted where necessary by increasing the doses of recombinant gonadotropins, according to the results of ovarian monitoring. In such groups of patients, personalizing the stimulation doses, the clinical outcome results achieved fluctuated from 39.5% to 88.5% PR per patient[10,58,61,66,69] (Table 2).

On the other hand, although the efficacy of using combined rFSH and rLH to stimulate HH patients has been proven, a few studies have reported on the possible side effects, such as local reaction, tolerability, and the risk of developing OHSS, when using recombinant rFSH/rLH, compared to conventional hMG, in the treatment of such a group of HH patients. A study published by Burguès et al[65] evaluated the safety of using rFSH/rLH combined protocol in the treatment of WHO type 1 anovulation patients, and they found that the risk of OHSS occurred in three out of 38 treated patients (one mild and two moderate OHSS cases). They also assessed 984 injections for local tolerance and observed that 9% (88/984) of injections were associated with local reactions; only 1.1% of injections were associated with severe pain and 0.5% with severe itching. Despite the lack of a control hMG group in this study, the authors concluded that the combined protocol of rFSH/rLH is well tolerated. Moreover, Carone et al[69] reported no adverse events, and no

Table 2 Clinical results with the use of rFSH and rLH in HH women

Study	Type of study	Number of patients	Number of cycles	Number of pregnancies	PR per patient (%)	PR per cycle (%)	PR per hCG (%)
Kousta et al[58]	Case report	1	2	1	100	50	50
Agrawal et al[10]	Case report	1	3	1	100	33	33
Campo et al[60]	Case report	1	1	1	100	100	100
European Recombinant Human LH Study Group,[63]	Prospective, randomized	38	53	–	–	20.29	–
Burgués et al[65]	Prospective, randomized	38	84	15	39.5	18	22
El-Shawarby et al[61]	Case report	1	2	1	100	50	50
Balasch and Fábregues[62]	Case report	1	1	1	100	100	100
Kaufmann et al[66]	Prospective, randomized	31	54	20	64	37	59.3
Shoham et al[67]	Prospective, randomized	27	27	2	8	8	15
O'Dea et al[68]	Prospective, randomized	15	–	3	20	–	–
Carone et al[69]	Prospective, randomized	17	27	15	88.2	55.5	–

Abbreviations: hCG, human chorionic gonadotropin; HH, hypogonadotropic hypogonadism; PR, pregnancy rate; rFSH, recombinant follicle-stimulating hormone; rLH, recombinant luteinizing hormone.

mild or moderate OHSS was observed in 35 patients, treated for a total of 70 cycles, following ovarian stimulation either with hMG-HP or rFSH/rLH. Other studies, however, reported a significantly increased incidence of OHSS risk in normogonadotropic patients stimulated with rFSH + rLH compared to patients treated with hMG.[80,81] Indeed, further comparative studies are warranted to investigate the tolerability, acceptability, and other adverse events, such as the risk of OHSS, on using rFSH/rLH to stimulate hypogonadotropic patients compared to the same using conventional hMG regimens.

"Iatrogenic" severe LH and FSH deficiency during assisted reproductive technology cycles

A peculiar form of HH could be relieved following controlled ovarian hyperstimulation treatment during assisted reproductive technology (ART) cycles. Ovarian stimulation protocols implement the administration of a GnRH agonist (GnRH-a) for pituitary desensitization, known as cycle downregulation phase. For many years, the GnRH-a long protocol has been considered the stimulation protocol of choice because of its ability to reversibly block pituitary function, thus preventing premature LH surge, by depletion of pituitary gonadotropin after an initial stimulatory phase. Exogenous gonadotropins are administered only when suppression of the hypothalamus–pituitary–gonad axis is achieved after administration of GnRH-a.[82] The degree of pituitary suppression depends also on the GnRH-a formulation as well as the mode and dose of administration.[83,84]

The administration of GnRH-a produces a sort of iatrogenic HH state.[12] As a consequence of pituitary postsuppression, a decline occurs in either FSH concentrations (ranged between 1.5 IU/L and 3.5 IU/L) or LH concentrations (ranged between 0.5 IU/L and 2 IU/L). In some cases, the LH concentration often decreases to $<$0.5 IU/L during the intermediate-to-late stages of stimulation, which is even less than the value observed in true HH.[12]

However, according to the previously reported "spare receptor hypothesis", androgen synthesis and release are optimal even with $<$1% of LH receptors occupied, allowing adequate multifollicular growth and maturation with the administration of FSH alone.[11] Moreover, in some normogonadotropic women, with normal ovarian reserve, using rFSH alone is not efficient in inducing appropriate multifollicular growth following pituitary GnRH downregulation.[84,85] Some authors attributed this phenomenon to the profound suppression of LH level after long downregulation GnRH-a protocol.[83,84] Several studies have been conducted with the aim of establishing a valuable cutoff of circulating LH level after pituitary desensitization in order to identify those patients, but the results obtained are conflicting.[84,85] Conversely, a meta-analysis published by Kolibianakis et al[86] showed no evidence that low endogenous LH levels, which may occur during long protocols of ovarian stimulation for in vitro fertilization, require exogenous LH supplementation to improve the probability of ongoing pregnancy.

Additionally, other studies have demonstrated that a subgroup of normogonadotropic women, who are "hyporesponsive" to rFSH monotherapy following GnRH-a downregulation, may benefit from rLH administration, irrespective of the basal LH levels, to achieve proper multifollicular growth.[87–90] In another study, Lisi et al[91] reported that using rLH and rFSH to stimulate patients who had a hyporesponse to rFSH alone in a previous downregulated cycle improves the fertilization rate (from 60.9% to 86%) and the clinical PR (from 5.9% to 50%).

After these preliminary reports, it clearly appears that LH levels after downregulation may not have predictive value in identifying hyporesponsive patients. This has induced other researchers to attempt other strategies to identify hyporesponsive patients during the early stimulation phase and to evaluate the possible use of rLH in the same cycle of stimulation, thus trying a sort of rescue of stimulation treatment.[87–90] The results were compared with those obtained in the controlled group of hyporesponsive patients, in which the rescue strategy was based on an increase of rFSH daily doses, and/or in normoresponsive patients.[87–90] In all these studies, different protocols were used to evaluate rLH doses, timing of rLH administration, and starting doses of rFSH. Primary and secondary end points evaluated differed in each of the reported studies; nevertheless, all observed results showed a beneficial effect of the administration of rLH in these iatrogenic HH subgroup patients. Ferraretti et al[88] report a statistically significant difference in terms of implantation rate (36.8% in rLH + rFSH group vs 14.1% in rFSH-only group) and PR (54% in rLH + rFSH group vs 24% in rFSH-only group) with a dose of 75 IU or 150 IU rLH daily.

Conversely, De Placido et al[87] found an increase in the number of oocytes retrieved and in the percentage of mature oocytes when 150 IU/d of rLH was added to stimulate the steady responder patients, as well as a statistically significant decrease in both variables when adding 75 IU/d of rLH. In another study, De Placido et al[89] evaluated the difference in the number and maturity of retrieved oocytes in a group of "steady responders" when 150 IU/d of rLH was added to rFSH compared to increased doses of rFSH without rLH supplementation.

As expected, in the group that received rLH in association with rFSH, the stimulation outcomes were comparable with those obtained in normal responders, whereas in the group of steady responders, who received only increased doses of rFSH, reductions in the total number of retrieved oocytes and in the total number of mature oocytes were observed.

More recently, Yazici Yilmaz et al[90] conducted a similar study, using 75 IU/d of rLH instead of 150 IU/d; they found no differences between the two subgroups of steady responders, while the total number of retrieved oocytes and the total number of mature oocytes were reduced as compared to those in normoresponsive patients. Interestingly, when considering implantation rate and PR, they observed similar results in the subgroup of patients supplemented with rLH compared to those observed in normoresponsive patients, whereas a statistically significant decrease was observed in terms of implantation rate and PR in the subgroup of steady responders who received only increasing doses of rFSH alone daily. These findings may be related to a discrepancy between the bioactive and immunoreactive forms of LH.[92–94] In some of these patients, the presence of a polymorphism in the LH beta subunit gene (the variant being termed v-betaLH), which affects ~10% of the population, may explain the hyporesponse to rFSH monotherapy.[95] Alternatively, ovarian resistance to rFSH has also been advocated as a possible cause for this hyporesponsivness.[96]

From the data in literature, it appears that the subgroup of hyporesponder patients needs LH activity (rLH or hMG) supplementation, instead of increased doses of rFSH, in order to achieve appropriate follicular growth, ovulation, and oocyte competence, as well as results comparable to those of the normoresponder patients in terms of PR.[12]

Conclusion

Severe LH and FSH deficiency is not a common finding among women. Although studies on this issue are limited and the experiences available are few due to the small number of such patients, it is clearly evident that the recombinant gonadotropins rFSH and rLH are efficient in treating patients affected by HH. The results observed in the studies reported in this review suggest that recombinant gonadotropins are able to induce appropriate follicular growth, oocyte maturation, and – eventually – pregnancy in this group of women. Moreover, the clinical use of recombinant gonadotropins in this type of patient has elucidated some endocrinological aspects of ovarian function, which have not yet been fully understood by using urinary gonadotropins. However, additional studies should seek to address the safety and efficiency of recombinant gonadotropins in ovarian stimulation and aim to heighten our knowledge on the physiological hormonal mechanism governing follicular growth and ovulation and, consequently, to improve the clinical applications of recombinant gonadotropins in fertility restoration.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Schoot DC, Coelingh Bennink HJ, Mannaerts BM, Lamberts SW, Bouchard P, Fauser BC. Human recombinant follicle-stimulating hormone induces growth of preovulatory follicles without concomitant increase in androgen and estrogen biosynthesis in a woman with isolated gonadotropin deficiency. *J Clin Endocrinol Metab*. 1992;74(6):1471–1473.
2. Schoot DC, Harlin J, Shoham Z, et al. Recombinant human follicle-stimulating hormone and ovarian response in gonadotrophin-deficient women. *Hum Reprod*. 1994;9(7):1237–1242.
3. Couzinet B, Lestrat N, Brailly S, Forest M, Schaison G. Stimulation of ovarian follicular maturation with pure follicle-stimulating hormone in women with gonadotropin deficiency. *J Clin Endocrinol Metab*. 1988;66(3):552–556.
4. Braat DD, Schoemaker J. Endocrinology of gonadotropin-releasing hormone induced cycles in hypothalamic amenorrhea: the role of the pulse dose. *Fertil Steril*. 1991;56(6):1054–1059.
5. Martin KA, Hall JE, Adams JM, Crowley WF Jr. Comparison of exogenous gonadotropins and pulsatile gonadotropin-releasing hormone for induction of ovulation in hypogonadotropic amenorrhea. *J Clin Endocrinol Metab*. 1993;77(1):125–129.
6. Skarin G, Ahlgren M. Pulsatile gonadotropin releasing hormone (GnRH): treatment for hypothalamic amenorrhea causing infertility. *Acta Obstet Gynecol Scand*. 1994;73(6):482–485.
7. Balen AH, Braat DD, West C, Patel A, Jacobs HS. Cumulative conception and live birth rates after the treatment of anovulatory infertility: safety and efficacy of ovulation induction in 200 patients. *Hum Reprod*. 1994;9(8):1563–1570.
8. Filicori M, Flamigni C, Dellai P, Cognigni G, Michelacci L, Arnone R. Treatment of anovulation with pulsatile gonadotropin-releasing hormone: prognostic factors and clinical results in 600 cycles. *J Clin Endocrinol Metab*. 1994;79(4):1215–1220.
9. Shoham Z, Balen A, Patel A, Jacobs HS. Results of ovulation induction using human menopausal gonadotropin or purified follicle-stimulating hormone in hypogonadotropic hypogonadism patients. *Fertil Steril*. 1991;56(6):1048–1053.
10. Agrawal R, West C, Conway GS, Page ML, Jacobs HS. Pregnancy after treatment with three recombinant gonadotropins. *Lancet*. 1997;349(9044):29–30.
11. Chappel SC, Howles C. Reevaluation of the roles of luteinizing hormone and follicle-stimulating hormone in the ovulatory process. *Hum Reprod*. 1991;6(9):1206–1212.
12. Alviggi C, Clarizia R, Mollo A, Ranieri A, De Placido G. Who needs LH in ovarian stimulation? *Reprod Biomed Online*. 2011;22(suppl 1):S33–S41.
13. Rama Raju GA, Chavan R, Deenadayal M, et al. Luteinizing hormone and follicle stimulating hormone synergy: a review of role in controlled ovarian hyper-stimulation. *J Hum Reprod Sci*. 2013;6(4):227–234.
14. Seminara SB, Hayes FJ, Crowley WF Jr. Gonadotropin-releasing hormone deficiency in the human (idiopathic hypogonadotropic hypogonadism and Kallmann's syndrome): pathophysiological and genetic considerations. *Endocr Rev*. 1998;19(5):521–539.
15. Silveira LF, Latronico AC. Approach to the patient with hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*. 2013;98(5):1781–1788.

16. Silveira LF, MacColl GS, Bouloux PM. Hypogonadotropic hypogonadism. *Semin Reprod Med*. 2002;20(4):327–338.
17. Gordon CM. Clinical practice. Functional hypothalamic amenorrhea. *N Engl J Med*. 2010;363(4):365–371.
18. Lapthorn AJ, Harris DC, Littlejohn A, et al. Crystal structure of human chorionic gonadotropin. *Nature*. 1994;369(6480):455–461.
19. Gibreel A, Bhattacharya S. Recombinant follitropin alfa/lutropin alfa in fertility treatment. *Biologics*. 2010;4:5–17.
20. Vaitukaitis JL, Ross GT, Braunstein GD, Rayford PL. Gonadotropins and their subunits: basic and clinical studies. *Recent Prog Horm Res*. 1976; 32:289–331.
21. Ulloa-Aguirre A, Espinoza R, Damian-Matsumura P, Chappel SC. Immunological and biological potencies of the different molecular species of gonadotrophins. *Hum Reprod*. 1988;3(4):491–501.
22. Practice Committee of American Society for Reproductive Medicine, Birmingham, Alabama. Gonadotropin preparations: past, present, and future perspectives. *Fertil Steril*. 2008;90(5 suppl):S13–S20.
23. Green ED, Baenziger JU. Asparagine-linked oligosaccharides on lutropin, follitropin, and thyrotropin. II. Distributions of sulfated and sialylated oligosaccharides on bovine, ovine, and human pituitary glycoprotein hormones. *J Biol Chem*. 1988;263(1):36–44.
24. Dahl KD, Stone MP. FSH isoforms, radioimmunoassays, bioassays, and their significance. *J Androl*. 1992;13(1):11–22.
25. Bishop LA, Robertson DM, Cahir N, Schofield PR. Specific roles for the asparagine-linked carbohydrate residues of recombinant human follicle-stimulating hormone in receptor binding and signal transduction. *Mol Endocrinol*. 1994;8(6):722–731.
26. Andersen AN, Devroey P, Arce JC. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: a randomized assessor-blind controlled trial. *Hum Reprod*. 2006;21(12):3217–3227.
27. Lambert A, Rodgers M, Mitchell R, et al. In-vitro biopotency and glycoform distribution of recombinant human follicle stimulating hormone (Org 32489), Metrodin and Metrodin-HP. *Hum Reprod*. 1995;10(7): 1928–1935.
28. Howles CM. Genetic engineering of human FSH (Gonal-F). *Hum Reprod Update*. 1996;2(2):172–191.
29. Olijve W, de Boer W, Mulders JW, van Wezenbeek PM. Molecular biology and biochemistry of human recombinant follicle stimulating hormone (Puregon). *Mol Hum Reprod*. 1996;2(5):371–382.
30. Alper M, Meyer R, Dekkers C, Ezcurra D, Schertz J, Kelly E. Assessment of the biopotency of follitropin alfa and lutropin alfa combined in one injection: a comparative trial in Sprague-Dawley rats. *Reprod Biol Endocrinol*. 2008;6:31–36.
31. Saz-Parkinson Z, López-Cuadrado T, Bouza C, Amate JM. Outcomes of new quality standards of follitropin alfa on ovarian stimulation: meta-analysis of previous studies. *BioDrugs*. 2009;23(1):37–42.
32. Dhillon S, Keating GM. Lutropin alfa. *Drugs*. 2008;68(11):1529–1540.
33. Pierce JG, Faith MR, Giudice LC, Reeve JR. Structure and structure-function relationships in glycoprotein hormones. *Ciba Found Symp*. 1976; 41:225–250.
34. Goa KL, Wagstaff AJ. Follitropin alfa in infertility: a review. *BioDrugs*. 1998;9(3):235–260.
35. Lunenfeld B. Historical perspectives in gonadotrophin therapy. *Hum Reprod Update*. 2004;10(6):453–467.
36. Anobile CJ, Talbot JA, McCann SJ, Padmanabhan V, Robertson WR. Glycoform composition of serum gonadotrophins through the normal menstrual cycle and in the post-menopausal state. *Mol Hum Reprod*. 1998;4(7):631–639.
37. Bergh C, Howles CM, Borg K, et al. Recombinant human follicle-stimulating hormone (r-FSH, Gonal-F) vs. highly purified urinary FSH (Metrodin highly purified): results of a randomized comparative study in women undergoing assisted reproductive techniques. *Hum Reprod*. 1997;12(10):2133–2139.
38. Al-Inany H, Aboulghar M, Mansour R, Gamal Serour G. Meta-analysis of recombinant versus urinary-derived FSH: an update. *Hum Reprod*. 2003;18(2):305–313.
39. Selman HA, De Santo M, Sterzik K, Coccia E, El-Danasouri I. Effect of highly purified urinary follicle-stimulating hormone on oocyte and embryo quality. *Fertil Steril*. 2002;78(5):1061–1067.
40. Strehler E, Abt M, El-Danasouri I, De Santo M, Sterzik K. Impact of recombinant follicle-stimulating hormone and human menopausal gonadotrophins on in vitro fertilization outcome. *Fertil Steril*. 2001;75(2):332–336.
41. West CR, Carlson NE, Lee JS, et al. Acidic mix of FSH isoforms are better facilitators of ovarian follicular maturation and E2 production than the less acidic. *Endocrinology*. 2002;143(1):107–116.
42. Wide L, Hobson BM. Influence of the assay method used on the selection of the most active forms of FSH from the human pituitary. *Acta Endocrinol*. 1986;113(1):17–22.
43. Ulloa-Aguirre A, Cravioto A, Damian-Matsumura P, Jimenez M, Zambrano E, Diaz-Sanchez V. Biological characterization of the naturally occurring analogues of intrapituitary human follicle-stimulating hormone. *Hum Reprod*. 1992;7(1):23–30.
44. Wide L. The regulation of metabolic clearance rate of human FSH in mice by variation of the molecular structure of the hormone. *Endocrinology*. 1986;112(3):336–344.
45. Lispi M, Bassett R, Crisci C, et al. Comparative assessment of the consistency and quality of a highly purified FSH extracted from urine (urofollitropin) and a recombinant human FSH (follitropin alpha). *Reprod Biomed Online*. 2006;13(2):179–193.
46. Blum WF, Gupta D. Heterogeneity of rat FSH by chromatofocusing: studies on serum FSH, hormone released in vitro and metabolic clearance rates of its various forms. *J Endocrinol*. 1985;105(1):29–37.
47. Bishop LA, Nguyen TV, Schofield PR. Both of the beta-subunit carbohydrate residues of follicle-stimulating hormone determine the metabolic clearance rate and in vivo potency. *Endocrinology*. 1995;136(6): 2635–2640.
48. Antonio MD, Borrelli F, Datola A, et al. Biological characterization of recombinant human follicle stimulating hormone isoforms. *Hum Reprod*. 1999;14(5):1160–1167.
49. Ulloa-Aguirre A, Damian-Matsumura P, Jimenez M, Zambarano E, Diaz-Sanchez V. Biological characterization of the isoforms of urinary human follicle stimulating hormone contained in a purified commercial preparation. *Hum Reprod*. 1992;7(10):1371–1378.
50. Flack MR, Bennet AP, Froehlich J, Anasti JN. Increased biological activity due to basic isoforms in recombinant human follicle stimulating hormone produced in a human cell line. *J Clin Endocrinol Metab*. 1994;79(3):756–760.
51. Galway AB, Hsueh AJ, Keene JL, Yamoto M, Fauser BC, Boime I. In vitro and in vivo bioactivity of recombinant human follicle-stimulating hormone and partially deglycosylated variants secreted by transfected eukaryotic cell lines. *Endocrinology*. 1990;127(1):93–100.
52. Nayudu LP, Vitt UA, Barrios DE, Tomasi J, Pancharatna K, Ulloa-Aguirre A. Intact follicle culture: what it can tell us about the role of FSH glycoforms during follicle development. *Reprod Biomed Online*. 2002;5(3):240–553.
53. Vitt UA, Nayudu PL, Rose UM, Kloosterboer HJ. Embryonic development after follicle culture is influenced by follicle stimulating hormone isoelectric point charge. *Biol Reprod*. 2001;65(5):1542–1547.
54. Selman H, Pacchiarotti A, El-Danasouri I. Ovarian stimulation protocols based on follicle-stimulating hormone glycosylation pattern: impact on oocyte quality and clinical outcome. *Fertil Steril*. 2010;94(5): 1782–1786.
55. Picard M, Rossier C, Papasouliotis O, Lugan I. Bioequivalence of recombinant human FSH and recombinant human LH in a fixed 2:1 combination: two phase I, randomised, crossover studies. *Curr Med Res Opin*. 2008;24(4):1199–1208.
56. Agostinetto R. Administration of follitropin alfa and luteotropin alfa combined in a single injection: a feasibility assessment. *Reprod Biol Endocrinol*. 2009;7:48–52.
57. Tourabizadeh A, Fatemeh VR. Evaluating the prevalence of hypogonadotropic amenorrhea in infertile women and the rate of pregnancy following treatment. *J Reprod Infertil*. 2005;6(3):247–253.

58. Kousta E, White DM, Piazzi A, Loumaye E, Franks S. Successful induction of ovulation and completed pregnancy using recombinant luteinizing hormone and follicle stimulating hormone in a woman with Kallmann's syndrome. *Hum Reprod*. 1996;11(1):70–71.
59. Shoham Z, Loumaye E, Piazzi A. A dose finding study to determine the effective dose of recombinant human luteinizing hormone to support FSH-induced follicular development in hypogonadotropic hypogonadal (HH) women. In: Proceedings of the 51st Annual Meet of the Am Soc for Reprod Med; October, 1995; Seattle, WA. Abstract S69–S70.
60. Campo S, Campo V, Lanzone A. Twin pregnancy using recombinant gonadotropins in a woman with hypogonadotropic hypogonadism. *Gynecol Endocrinol*. 2002;16(1):27–32.
61. El-Shawarby SA, Turner CF, Reddy N, Margara RA, Trew GH, Lavery SA. Pregnancy following monofollicular ovulation induction with recombinant FSH, recombinant LH and timed coitus in an amenorrheic woman with long-standing hypogonadotrophic hypogonadism. *BJOG*. 2004;111(12):1481–1484.
62. Balasch J, Fábregues F. Pregnancy after administration of high dose recombinant human LH alone to support final stages of follicular maturation in a woman with long-standing hypogonadotrophic hypogonadism. *Reprod Biomed Online*. 2003;6(4):427–431.
63. The European Recombinant Human LH Study Group. Recombinant human luteinizing hormone (LH) to support recombinant human follicle-stimulating hormone (FSH)-induced follicular development in LH- and FSH-deficient anovulatory women: a dose-finding study. *J Clin Endocrinol Metab*. 1998;83(5):1507–1514.
64. Loumaye E, Engrand P, Shoham Z, Hillier SG, Baird DT. Clinical evidence for an LH 'ceiling' effect induced by administration of recombinant human LH during the late follicular phase of stimulated cycles in World Health Organization type I and type II anovulation. *Hum Reprod*. 2003;18(2):314–322.
65. Burgués S; Spanish Collaborative Group on Female Hypogonadotrophic Hypogonadism. The effectiveness and safety of recombinant human LH to support follicular development induced by recombinant human FSH in WHO group I anovulation: evidence from a multicentre study in Spain. *Hum Reprod*. 2001;16(12):2525–2532.
66. Kaufmann R, Dunn R, Vaughn T, et al. Recombinant human luteinizing hormone, lutropin alfa, for the induction of follicular development and pregnancy in profoundly gonadotrophin-deficient women. *Clin Endocrinol (Oxf)*. 2007;67(4):563–569.
67. Shoham Z, Smith H, Yeko T, O'Brien F, Hemsey G, O'Dea L. Recombinant LH (lutropin alfa) for the treatment of hypogonadotrophic women with profound LH deficiency: a randomized, double-blind, placebo-controlled, proof-of-efficacy study. *Clin Endocrinol (Oxf)*. 2008;69(3):471–478.
68. O'Dea L, O'Brien F, Currie K, Hemsey G. Follicular development induced by recombinant luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in anovulatory women with LH and FSH deficiency: evidence of a threshold effect. *Curr Med Res Opin*. 2008; 24(10):2785–2793.
69. Carone D, Caropreso C, Vitti A, Chiappetta R. Efficacy of different gonadotropin combinations to support ovulation induction in WHO type I anovulation infertility: clinical evidences of human recombinant FSH/human recombinant LH in a 2:1 ratio and highly purified human menopausal gonadotropin stimulation protocols. *J Endocrinol Invest*. 2012;35(11):996–1002.
70. Basset R, Lispi M, Ceccarelli D, et al. Analytical identification of additional impurities in urinary-derived gonadotrophins. *Reprod Biomed Online*. 2009;19(3):300–313.
71. Dickinson RE, Stewart AJ, Myesrs M, Milar RP, Duncan WC. Differential expression and functional characterization of luteinizing hormone receptor splice variants in human luteal cells: implications for luteolysis. *Endocrinology*. 2009;150(6):2873–2881.
72. Filicori M, Fazleabas AT, Huhtaniemi I, et al. Novel concepts of human chorionic gonadotropin: reproductive system interactions and potential in the management of infertility. *Fertil Steril*. 2005;84(2):275–284.
73. Grøndahl ML, Borup R, Lee YB, Myrhøj V, Meinertz H, Sørensen S. Differences in gene expression of granulosa cells from women undergoing controlled ovarian hyperstimulation with either recombinant follicle-stimulating hormone or highly purified human menopausal gonadotropin. *Fertil Steril*. 2009;91(5):1820–1830.
74. Menon KM, Munshi UM, Clouser CL, Nair AK. Regulation of luteinizing hormone/human chorionic gonadotropin receptor expression: a perspective. *Biol Reprod*. 2004;70(4):861–866.
75. Wong PC, Qiao J, Ho C, et al; Asia Pacific Fertility Advisory Group. Current opinion on use of luteinizing hormone supplementation in assisted reproduction therapy: an Asian perspective. *Reprod Biomed Online*. 2011;23(1):81–90.
76. Menon KM, Nair AK, Wang L. A novel post-transcriptional mechanism of regulation of luteinizing hormone receptor expression by an RNA binding protein from the ovary. *Mol Cell Endocrinol*. 2006; 246(1–2):135–141.
77. Papaleo E, Alviggi C, Colombo GL, et al. Cost-effectiveness analysis on the use of rFSH + rLH for the treatment of anovulation in hypogonadotropic hypogonadal women. *Ther Clin Risk Manag*. 2014;10: 479–484.
78. Awwad JT, Farra C, Mitri F, Abdallah MA, Jaoudeh MA, Ghazeeri G. Split daily recombinant human LH dose in hypogonadotrophic hypogonadism: a nonrandomized controlled pilot study. *Reprod Biomed Online*. 2013;26(1):88–92.
79. Hull M, Corrigan E, Piazzi A, Loumaye E. Recombinant human luteinising hormone: an effective new gonadotropin preparation. *Lancet*. 1994;344(8918):334–335.
80. Pacchiarotti A, Sbracia M, Frega A, Selman H, Rinaldi L, Pacchiarotti A. Urinary hMG (Meropur) versus recombinant FSH plus recombinant LH (Pergoveris) in IVF: a multicenter, prospective, randomized controlled trial. *Fertil Steril*. 2010;94(6):2467–2469.
81. Moro F, Scarinci E, Palla C, et al. Highly purified hMG versus recombinant FSH plus recombinant LH in intrauterine insemination cycles in women ≥35 years: a RCT. *Hum Reprod*. 2015;30(1):179–185.
82. Hughes EG, Fedorkow DM, Daya S, Sagle MA, Van de Koppel P, Collins JA. The routine use of gonadotrophin-releasing hormone agonists prior to in vitro fertilisation and gamete intrafallopian transfer: a meta-analysis of randomised controlled trials. *Fertil Steril*. 1992;58(5): 888–896.
83. Sonntag B, Kiesel L, Nieschlag E, Behre HM. Differences in serum LH and FSH levels using depot or daily GnRH agonists in controlled ovarian stimulation: influence on ovarian response and outcome of ART. *J Assist Reprod Genet*. 2005;22(7–8):277–283.
84. Westergaard LG, Laursen SB, Andersen CY. Increased risk of early pregnancy loss by profound suppression of luteinizing hormone during ovarian stimulation in normogonadotrophic women undergoing assisted reproduction. *Hum Reprod*. 2000;15(5):1003–1008.
85. Balasch J, Vidal E, Peñarrubia J, et al. Suppression of LH during ovarian stimulation: analysing threshold values and effects on ovarian response and the outcome of assisted reproduction in down-regulated women stimulated with recombinant FSH. *Hum Reprod*. 2001;16(8): 1636–1643.
86. Kolibianakis EM, Collins J, Tarlatzis B, Papanikolaou E, Devroey P. Are endogenous LH levels during ovarian stimulation for IVF using GnRH analogues associated with the probability of ongoing pregnancy? A systematic review. *Hum Reprod Update*. 2006;12(1): 3–12.
87. De Placido G, Alviggi C, Mollo A, et al. Effects of recombinant LH (rLH) supplementation during controlled ovarian hyperstimulation (COH) in normogonadotrophic women with an initial inadeguate response to recombinant FSH (rFSH) after pituitary downregulation. *Clin Endocrinol (Oxf)*. 2004;60:637–643.
88. Ferraretti AP, Gianaroli L, Magli MC, D'angelo A, Farfalli V, Montanaro N. Exogenous luteinizing hormone in controlled ovarian hyperstimulation for assisted reproduction techniques. *Fertil Steril*. 2004;82(6): 1521–1526.

89. De Placido G, Alviggi C, Perino A, Strina I, Lisi F; Italian Collaborative Group on Recombinant Human Luteinizing Hormone. Recombinant human LH supplementation versus recombinant human FSH (rFSH) step-up protocol during controlled ovarian stimulation in normogonadotrophic women with initial inadequate ovarian response to rFSH. A multicentre, prospective, randomized controlled trial. *Hum Reprod*. 2005;20:390–396.
90. Yazıcı Yılmaz F, Görkemli H, Çolakoğlu MC, Aktan M, Gezginç K. The evaluation of recombinant LH supplementation in patients with suboptimal response to recombinant FSH undergoing IVF treatment with GnRH agonist down-regulation. *Gynecol Endocrinol*. 2015;31(2):141–144.
91. Lisi F, Rinaldi L, Fishel S, et al. Use of recombinant FSH and recombinant LH in multiple follicular stimulation for IVF: a preliminary study. *Reprod Biomed Online*. 2001;3(3):190–194.
92. Huhtaniemi I, Jiang M, Nilsson C, Pettersson K. Mutations and polymorphisms in gonadotropin genes. *Mol Cell Endocrinol*. 1999;151(1–2):89–94.
93. Jiang M, Pakarinen P, Zhang FP, et al. A common polymorphic allele of the human luteinizing hormone beta-subunit gene: additional mutations and differential function of the promoter sequence. *Hum Mol Genet*. 1999;8(11):2037–2046.
94. Ropelato MG, Garcia-Rudaz MC, Castro-Fernandez C, et al. A preponderance of basic luteinizing hormone (LH) isoforms accompanies inappropriate hypersecretion of both basal and pulsatile LH in adolescents with polycystic ovarian syndrome. *J Clin Endocrinol Metab*. 1999;84(12):4629–4636.
95. Alviggi C, Pettersson K, Longobardi S, et al. A common polymorphic allele of the LH beta-subunit gene is associated with higher exogenous FSH consumption during controlled ovarian stimulation for assisted reproductive technology. *Reprod Biol Endocrinol*. 2013;11:51.
96. Alviggi C, Pettersson K, Mollo A, et al. Impaired multiple follicular development in carriers of Trp8Arg and Ile15Thr LLbeta variant undergoing controlled ovarian stimulation. *Human Reprod*. 2005;20(suppl 1):i139.