OBJECTIVE: To present an evidence-based rationale for or against the use of supplemental luteinizing hormone (LH) versus follicle-stimulating hormone (FSH) alone in various controlled ovarian stimulation (COS) protocols and specific patient populations. Any evidence for or against an association between endogenous LH levels and the likelihood of pregnancy in in vitro fertilization (IVF) procedures will also be presented.

STUDY DESIGN: Literature review and critical analysis of evidence drawn from systematic reviews and meta-analyses published in the last 3 years and from data subsequently reported (through mid-2009) by well-controlled randomized trials. Evidence from observational and retrospective studies will be discussed if no higher-level evidence is available on a particular topic.

RESULTS: Low endogenous LH levels associated with long gonadotropin-releasing hormone (GnRH) agonist protocols do not appear to decrease the probability of a successful IVF outcome. Supplemental LH does not appear to be routinely indicated for patients undergoing COS in long GnRH agonist protocols, though the evidence seems to favor some form of LH add-back in certain well-defined subgroups treated with these protocols.

CONCLUSION: Current evidence suggests that exogenous LH does not confer any significant benefit in women undergoing treatment with GnRH antagonist regimens, but more data are needed to confirm this finding. (J Reprod Med 2011; 56:279–300)

Keywords: FSH, human; GnRH; gonadotropins, human menopausal; in vitro fertilization; intracytoplasmic sperm injections; LH (luteinizing hormone).

Luteinizing hormone (LH) plays a well-defined role in the normal menstrual cycle, but it is not yet clear whether exogenously supplemented LH activity exerts a positive effect on follicular maturation during controlled ovarian stimulation (COS) and the likelihood of pregnancy after in vitro fertilization (IVF). Some of the controversy surrounding the use or nonuse of supplemental LH in COS arises from differences in the methodology employed by the various studies that have explored this issue. Differences in defined study objectives and established clinical end points may partially account for reported disparities in the findings.
Physiologic Role of LH in the Normal Cycle

The normal menstrual cycle is regulated by the complex interaction of FSH, LH and the sex steroids estrogen and progesterone (Figure 1).1,2 The “two-cell, two-gonadotropin” model of folliculogenesis proposes that both FSH and LH are required for the growth and maturation of the ovarian follicle.3 According to this model, granulosa and theca cells interact to regulate estrogen secretion by the ovary and the preovulatory development of the ovarian follicle. FSH acts directly on granulosa cells to stimulate the activity of aromatase, the enzyme that converts androgens to estrogens, while LH promotes the production of androgens by theca cells. Thus, adequate biosynthesis of estradiol requires sufficient stimulation of both theca cells by LH and of granulosa cells by FSH.3

In addition to regulating estradiol biosynthesis, LH also appears to play another key role in folliculogenesis.4 In the intermediate follicular phase, LH induces the production of various glycoproteins and polypeptides that promote the development of granulosa cells and exert endocrine effects on the pituitary and autocrine/paracrine effects on ovarian function. For example, there is evidence that LH directly controls the pattern of inhibin A secretion in mature follicles,5 and both LH and FSH induce the local production of inhibin B and insulin-like growth factors (IGF-I and IGF-II). A number of animal and in vitro studies have provided additional evidence of the complex interplay of endocrine, paracrine, and autocrine effects that appear to regulate the normal reproductive cycle (Table I).6–10

Data from these experiments confirm that concomitant LH stimulation is required for FSH-induced paracrine signaling to occur. In the complete absence of LH (e.g., in hypophysectomized animals), FSH alone cannot stimulate paracrine signaling in order to promote thecal androgen synthesis via up-regulation of mRNA for cytochrome P450 17α-hydroxylase (P450C17).9 However, when secretion of inhibin B and IGF-I is adequate, < 1% of the LH receptors need to be occupied to elicit a maximal steroidogenic response by thecal cells.11 Because of such paracrine effects, low levels of LH can apparently suffice to stimulate androgen synthesis in the preovulatory period.11

LH may play a third role in folliculogenesis by affecting the selection of the dominant follicle and promoting the degeneration of small follicles. Evidence from preclinical studies suggests that developing ovarian follicles have certain limited requirements for LH exposure, above which normal maturation ceases.2 It has been proposed that each follicle has an LH ceiling below which LH stimulation must occur in order to promote normal follicular growth.2 According to this LH ceiling hypothesis, the dominant follicles possess a higher LH ceiling than the more immature ones. An increasing concentration of LH during the second half of the follicular phase would thus foster the development of the dominant follicle (because the LH level is below its ceiling) but lead to growth arrest in the secondary follicles (by exceeding their LH ceiling). The effect of LH in arresting follicular growth becomes evident during the mid-cycle LH surge. After LH binds to receptors on the granulosa cells, granulosa cell mitosis ceases, meiosis resumes in the arrested oocyte, and functional and structural changes occur in cumulus oophorus cells prior to ovulation.12

It is now known that LH receptors are not exclusively expressed on theca cells. In the late follicular phase, FSH in the presence of estradiol stimulates the expression of LH receptors on the granulosa cells as well.2,13 Once the granulosa cells express the LH receptor, they can respond to LH, and at this
In conclusion, LH has been shown to play several important roles in the normal menstrual cycle. It acts directly on ovarian thecal cells to stimulate the synthesis of androgens, which are then converted to estradiol in the granulosa cells. LH, along with FSH, also induces the production of factors such as inhibins and insulin-like growth factors that exert endocrine effects on the pituitary as well as autocrine and paracrine effects on ovarian function. In addition, a rising LH level during the mid- to late-follicular phase may affect the selection of the dominant follicle while promoting the degeneration of small follicles. Finally, the steep mid-cycle rise, or surge, in LH triggers ovulation.

Is There an Association Between Endogenous LH and Clinical Outcome of IVF?

The role of LH in the normal reproductive cycle raises the question of whether the low endogenous LH levels that can occur during pituitary suppression with GnRH analogues are sufficient to foster complete maturation of the follicle and development of oocyte competence, or whether exogenous...
LH supplementation may be needed in some cases. Several studies have assessed what impact, if any, preovulatory endogenous LH levels may exert on ovarian response and ongoing pregnancy rates in women undergoing COS. This question has been examined for both GnRH agonist and GnRH antagonist protocols and in different patient populations, such as hypogonadotropic patients, “low responders,” and “normal responders.”

Various methodologies have been used to examine the possible associations between endogenous LH levels and clinical outcomes in IVF.\textsuperscript{14–23} Such methodologies have included observational data from interventional studies as well as prospective and retrospective observational studies; studies in which populations were typically stratified according to LH levels, using either arbitrary cutoffs or population-specific (e.g., decile-determined) cutoffs; studies employing receiver operating characteristic analysis to determine LH cutoff levels that might be predictive of clinical outcomes; and multivariate analyses that have included LH levels as well as other predictor variables in the equation.

Table I  \textit{Data from Preclinical Studies about Endocrine, Paracrine and Autocrine Influences in Normal Reproductive Cycle}

<table>
<thead>
<tr>
<th>Reference</th>
<th>Type of study</th>
<th>Study objective</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fortune et al\textsuperscript{6}</td>
<td>In vitro culture of theca and granulosa isolated from rat ovarian follicles</td>
<td>To identify cellular source and hormonal control of androgen secretion by rat ovarian follicles</td>
<td>Theca cells are the site of follicular androgen production, LH regulates androgen secretion and theca cells provide the androgen precursor needed for synthesis of estradiol-17β</td>
</tr>
<tr>
<td>Camp et al\textsuperscript{7}</td>
<td>Animal study performed in both immature female rats treated with gonadotropins and adult female rats that had regular 4-day estrous cycles</td>
<td>To examine expression and hormonal regulation of ovarian FSH and LH receptor mRNAs in the rat</td>
<td>In immature follicles, low levels of FSH and LH receptor mRNA were found in granulosa and theca cells, respectively; after stimulation with gonadotropin, LH receptor mRNA was found in both granulosa and theca cells of large follicles; later in the estrous cycle, LH receptor mRNA appeared in granulosa cells, interstitial tissues and corpora lutea</td>
</tr>
<tr>
<td>Smyth et al\textsuperscript{8}</td>
<td>Isolation and culture of thecal/interstitial cells from intact or hypophysectomized female Wistar rats</td>
<td>To obtain evidence of FSH-stimulated paracrine signaling in the ovary</td>
<td>Inhibin potently enhances LH-responsive androgen synthesis; FSH modulates thecal/interstitial cell androgen synthesis; granulosa cells (but not thecal cells) possess FSH receptors, and thecal/interstitial cells are the principal sites of expression of P450C17 (the LH-regulated enzyme required for androgen synthesis)</td>
</tr>
<tr>
<td>Hillier et al\textsuperscript{9}</td>
<td>Hypophysectomized, immature female rats were treated with rFSH and/or rLH; ovaries were removed and used to isolate granulosa and thecal interstitial cells for additional experiments</td>
<td>To assess how LH contributes to optimizing ovarian responsiveness to FSH</td>
<td>rFSH has potential to influence LH-responsive androgen synthesis (via a paracrine mechanism), which involves an upregulation of thecal P450C17 mRNA</td>
</tr>
<tr>
<td>Vänttinen et al\textsuperscript{10}</td>
<td>Primary cell culture–human ovarian granulosa-luteal cells</td>
<td>To evaluate endocrine and paracrine/autocrine regulation of inhibin A and inhibin B secretion in granulosa-luteal cells</td>
<td>Gonadotropins (FSH, LH, hCG) are main positive regulators of inhibin A and inhibin B secretion in human granulosa-luteal cells</td>
</tr>
</tbody>
</table>

FSH = follicle-stimulating hormone, hCG = human chorionic gonadotropin, LH = luteinizing hormone, mRNA = messenger RNA, rLH = recombinant luteinizing hormone.
etc.) were chosen to assess the association between endogenous LH and clinical outcome. In addition to assorted end points and the small size of most patient populations in these trials, study protocols differ on such variables as the type, timing and dose of the GnRH analogue, dose of FSH, establishment of LH thresholds and assay methods, and criteria for triggering final oocyte maturation.

Pituitary downregulation in long GnRH agonist protocols suppresses LH and FSH levels to varying degrees, depending on the type and dose of agonist being administered. In addition, the widespread replacement of human menopausal gonadotropin (hMG), which contains nearly equal amounts of FSH and LH activity, with pure FSH preparations (e.g., highly purified urinary FSH or recombinant FSH [rFSH]) devoid of LH activity could potentially have an impact on embryo developmental competence in women undergoing IVF treatment.

“Low” Versus “High” Endogenous LH Levels

In women with hypogonadotropic hypogonadism, the absence of significant amounts of LH adversely affects normal follicular maturation and potential pregnancy. Conversely, excessive follicular-phase LH levels, particularly at the time of maximum follicular growth, as often occur in women with polycystic ovary syndrome (PCOS), may have a deleterious effect on rates of conception and may contribute to high rates of early pregnancy loss. It has been hypothesized that high follicular-phase concentrations of LH may allow the hormone to penetrate the follicle, inducing premature maturation and subsequent ovulation of an “aged” oocyte.

Further, the PCOS syndrome is associated with excess androgen production. As IGF-I and inhibitin synergize with circulating levels of LH to stimulate androgen production by thecal cells, hyperstimulation and overproduction of androgen precursors occur. This subsequently leads to heightened secretion of ovarian estradiol from the granulosa cells, which may impair regular follicular development and the ovulatory process.

GnRH Agonist Protocols

Evidence from Recent Meta-analysis

A 2006 systematic review of the published literature extracted data from 6 studies of 1,103 patients treated with assisted reproductive technology (ART) procedures with the primary objective of examining the potential association of low endogenous LH levels with a significantly decreased probability of ongoing pregnancy after pituitary suppression with a GnRH analogue in normo-ovulatory or World Health Organization (WHO) II oligoanovulatory women. Included in this meta-analysis were 4 studies employing a GnRH agonist. The authors based their meta-analysis on the premise that a single outcome measure, i.e., live birth, or its near equivalent, ongoing pregnancy beyond 12 weeks of gestation, rather than surrogate outcome measures, should serve as the basis for determining whether endogenous LH concentrations can affect or predict IVF outcome in normo-ovulatory or WHO II patients. Only studies in which ovarian stimulation was performed in a single cycle using LH-free gonadotropins and pituitary LH suppression starting in either the luteal or follicular phase were included.

Westergaard et al performed a retrospective study of 200 normogonadotropic women downregulated with the GnRH agonist buserelin and stimulated with rFSH. They established a threshold value of 0.5 IU/L on day 8 to discriminate between women with “low” (< 0.5 IU/L) and “normal” (≥ 0.5 IU/L) serum LH concentrations. In 49% of patients, day 8 LH levels were < 0.5 IU/L, and in the remaining 51% they were ≥ 0.5 IU/L. They found a 5-fold higher risk of early pregnancy loss (p < 0.005) in the “low” versus the “normal” LH group. The percent of positive pregnancy tests per started cycle was similar between the 2 groups, however, and the difference in number of deliveries per clinical pregnancy did not achieve statistical significance.

The study by Balasch et al examined the impact of serum LH levels on day 7 of stimulation on the clinical outcome of 144 women undergoing IVF/ICSI after pituitary suppression with a GnRH agonist and stimulation with rFSH. When they examined the effect of 3 different mid-follicular LH levels that had been proposed to distinguish between “low” and “normal” endogenous LH values (< 0.5, ≤ 0.7, < 0.5 IU/L), they found no significant differences in ovarian response, IVF outcome, implantation and pregnancy outcome between women with “low” or “normal” LH levels as defined by these threshold values.

Humaidan and colleagues collected blood samples from 207 normogonadotropic women undergoing downregulation with the GnRH agonist buserelin and stimulation with rFSH for subsequent analysis of endogenous LH levels. Based on their median endogenous LH levels on stimulation day 8, the patients were divided into 4 groups: < 0.5,
0.51–1.00, 1.01–1.50 and > 1.5 IU/L. Although women with the highest LH level produced the highest levels of estradiol and estradiol per oocyte retrieved, the frequency of fertilization in this group was significantly lower than that in the 2 middle groups. In addition, the clinical pregnancy rate was significantly higher in the 0.51–1.0 IU/L LH group compared with the > 1.5 IU/L group.16

The results of this study indicate that circulating LH levels on day 8 that are either too low (<0.5 IU/L) or too high (>1.5 IU/L) may adversely impact the likelihood of a successful IVF outcome. The decreased pregnancy rates in those with high pre-ovulatory LH concentrations suggest that the LH ceiling, the threshold above which excessive exposure to the hormone can adversely affect oocyte development, may be relatively low.16 Interestingly, fewer than 12% of the participants in this study had serum LH levels <0.5 IU/L on day 8, a significantly lower percentage than the 49% reported in the earlier study by Westergaard et al.14

A study by Esposito et al17 assessed the possible impact of endogenous LH concentrations in the late-follicular phase on rates of fertilization, pregnancy and pregnancy loss in 166 normogonadotropic patients undergoing ART with luteal-phase leuprolide and fixed-dose rFSH stimulation. Patients were stratified according to an LH cutoff volume of 3 mIU/mL, accepted as the observed lower limit in natural cycles. Women with a mean LH concentration of <3 mIU/mL in the late-follicular phase (70%) experienced significantly lower rates of fertilization compared with those who had mean LH levels ≥3 mIU/mL (p = 0.03). Clinical pregnancy rates, implantation rates and spontaneous abortion rates, however, did not differ to a statistically significant degree between the 2 groups.17

Evidence from Subsequent Trials

A 2005 large, retrospective study by Bjerke et al18 that was published after trials had been selected for the meta-analysis by Kolibianakis et al29 assessed the potential usefulness of serum LH measurement on stimulation day 1 (prior to administration of rFSH) as a predictor of ovarian response, conception and pregnancy outcome in patients treated with a mid-luteal phase GnRH agonist protocol. When the records of 2,625 cycles in 1,652 women undergoing COS were reviewed, it was found that when cycles were stratified according to serum LH concentration on stimulation day 1, no significant association between endogenous LH levels and treatment outcome was observed. Thus, LH concentrations prior to administration of rFSH neither predicted pregnancy nor the ultimate clinical outcome in IVF/ICSI cycles stimulated with rFSH in a long GnRH agonist protocol.18

Another recent retrospective analysis sought to develop an index that could be used to predict ART outcomes and identify those women who might benefit from exogenous LH during COS.19 Serum LH concentrations were measured during the mid- and late-follicular phase in 86 normogonadotropic women who underwent IVF/ICSI using a long GnRH agonist protocol with rFSH. Treatment cycles were stratified into 3 groups based on LH concentrations (high: ≥1.2 IU/L; intermediate: ≥0.6 IU/L but <1.2 IU/L; and low: <0.6 IU/L). Late-follicular to mid-follicular ratios of <1.0 and ≥1.0 distinguished the relatively decreased (RD) LH group from the relatively increased (RI) LH group, respectively. The pregnancy (9.7%) and implantation (5.8%) rates in the RD group were significantly lower than those in the RI group (31.1% and 17.2%, respectively [p < 0.05 for both]). The analysis suggested that ART outcomes could not be predicted by serum LH concentrations measured at a single point in time. Rather, a late-follicular to mid-follicular LH ratio of <1.0, which represents the change in LH concentration throughout phases of ovarian stimulation, may be a more efficient index for identifying those ART patients who might benefit from LH supplementation in a long GnRH agonist protocol.19 Although these findings are intriguing, the number of subjects in this study (86 patients) is too small to permit any definitive interpretation by means of LH subset analyses.

Conclusions

The available evidence summarized in the meta-analysis of Kolibianakis et al29 suggests that low endogenous LH levels that occur during ovarian stimulation for IVF using long GnRH agonist protocols are not associated with a decreased probability of ongoing pregnancy beyond 12 weeks. The large, retrospective study by Bjerke et al18 in 2005 found no evidence that endogenous LH levels on stimulation day 1 were associated with the ultimate clinical outcome in patients treated with a long GnRH agonist protocol. Some data indicate that LH levels that are very high (>1.5 IU/L) on stimulation day 8 may be associated with a decreased probability of a successful IVF outcome.16 The association of endogenous serum LH with clinical outcome has been as-
sessed in only a few studies, however, so there is an unmet need for data from additional trials that may reinforce, refute or modify the conclusions drawn from the systematic review by Kolibianakis et al.

**GnRH Antagonist Protocols**

GnRH antagonists rapidly and reversibly suppress pituitary gonadotropin secretion by competitively binding to the GnRH receptor, inhibiting signal transduction and thereby preventing the LH surge observed with GnRH agonists. This mechanism of action causes immediate decline of endogenous LH levels within hours of administration. It has been suggested that such rapid and significant LH suppression during the mid-follicular phase of COS may adversely influence IVF outcomes with respect to oocyte yield, fertilization rates and available embryos for cryopreservation without affecting their developmental competence.30

A widespread problem in studies assessing LH levels after antagonist administration is that a lack of consideration is given to the amount of time elapsed between administration of the GnRH antagonist and the actual measurement of serum LH levels. Unlike studies using agonists, in which LH levels will fluctuate only slightly, LH levels can rise noticeably in multiple-dose regimens in the hours just prior to the next dose. Thus, without taking this factor into account, it is difficult to draw any broad conclusions about LH levels and antagonist cycle outcomes.31

**Evidence from Meta-analyses**

Two trials20,21 evaluating the potential impact of endogenous LH levels in patients undergoing pituitary suppression in GnRH antagonist protocols with rFSH stimulation were included in the recent meta-analysis by Kolibianakis et al.29

In a retrospective study of 270 normogonadotropic women undergoing COS with fixed-dose rFSH in a single-dose GnRH antagonist protocol, Merviel et al21 assessed the potential impact of circulating LH concentrations on ovarian response, pregnancy rates and embryo transfer outcome. Blood samples were collected on the day of GnRH antagonist administration, one day after and on the day of human chorionic gonadotropin (hCG). A threshold LH concentration of 0.5 IU/L on day of hCG was used to discriminate between 2 groups: women with levels ≤ 0.5 IU/L and those with LH levels > 0.5 IU/L.

In women with LH levels ≤ 0.5 IU/L on the day of hCG, the numbers of oocytes retrieved, embryos obtained and embryos cryopreserved were significantly higher compared with those whose LH levels were > 0.5 IU/L.21 However, the duration of ovarian stimulation, clinical pregnancy rate per transfer and take-home baby rates did not differ to a statistically significant degree between the 2 groups. These results indicate that in a single-dose GnRH antagonist protocol with rFSH stimulation, suppressed serum LH concentrations, i.e., ≤ 0.5 IU/L, on the day after GnRH antagonist administration and on day of hCG do not adversely influence fertilization rates, embryo viability, pregnancy rates or overall clinical outcomes.21

The other study included in the meta-analysis was a prospective clinical trial in which Kolibianakis et al20 evaluated the potential correlation between LH levels and ongoing pregnancy in 116 IVF patients treated with a fixed dose of rFSH in a GnRH antagonist protocol. Hormonal assessments for serum LH, FSH, hCG, estradiol and progesterone were performed on days 1, 6 and 8 of rFSH stimulation and on the day of hCG administration. LH levels of < 1.0 IU/L and ≤ 0.5 IU/L occurred in 54% and 21.6% of the patients studied, respectively. No differences in fertilization rates, the number and quality of embryos transferred, the quality of embryos cryopreserved or the type of embryo transfer performed were observed in groups with different percentiles of LH concentration. However, a significant decrease of both ongoing pregnancy rate and implantation rate was reported across groups of patients with increasing LH levels.20 The highest implantation rate (39.1%) and ongoing pregnancy rate (56.0%) occurred among those patients in the lowest percentile of day 8 LH concentration, i.e., between 0.10 IU/L and 0.50 IU/L. The higher the serum concentration of LH on COS day 8, the lower the probability of achieving an ongoing pregnancy after oocyte retrieval.20

**Evidence from Subsequent Trials**

Huirne et al22 recently assessed the optimal range of LH concentrations for achieving pregnancy in a prospective study of 144 IVF patients randomized to different doses of an experimental GnRH antagonist. The patients were stimulated with rFSH from cycle day 2 and co-treated with daily GnRH antagonist in 5 different doses (2 mg/2 mL, 1 mg/mL, 0.5 mg/mL, 0.5 mg/0.5 mL or 0.25 mg/mL) from cycle day 7 onward. One to 3 months prior to randomization, serum samples were obtained on cycle day...
2 or 3 for analysis of FSH, LH, estradiol, progesterone and prolactin; again on stimulation day 1 prior to injection of rFSH; and thereafter 3 times daily during GnRH antagonist administration.22

As expected, the highest total amount of LH secreted corresponded to the lowest serum GnRH antagonist levels, while the lowest LH values were associated with the highest GnRH antagonist values. The investigators found that clinical pregnancies occurred only within a specific range of change in LH levels. The upper and lower thresholds for the mean LH area under the curve (AUC), adjusted for the baseline LH level before the antagonist was started (LH AUC-S6; S6 = stimulation day 6), were −2.2 and 12.4 IU/L, respectively (a negative value indicates below baseline levels). No clinical pregnancies were observed above or below these threshold values. As with serum LH, a clear upper threshold for progesterone was detected at 0.26 ng/mL/follicle. No pregnancies occurred in patients with progesterone levels above this threshold on the day of hCG. These data suggest that either excessive or insufficient suppression of LH and progesterone levels during GnRH antagonist administration and high levels of progesterone per follicle on the day of hCG administration are associated with lower probabilities of clinical pregnancy.22

Bosch et al23 conducted a prospective cohort study to evaluate the effect of a multiple-dose GnRH antagonist protocol with rFSH stimulation on endogenous LH levels and to assess whether there was any correlation between low, medium and high LH concentrations in early, mid and late phases of COS and cycle outcome. Serum LH levels were determined on days 3, 6 and 8 and on the day of hCG administration in 110 young normogonadotropic women undergoing their first IVF cycle. Patients were stratified into 3 groups according to percentile of LH concentration: one group with low LH levels (below the 25th percentile), one with medium LH levels (between the 25th and 75th percentiles) and one with high LH levels (above the 75th percentile). No differences among the groups were observed between the number of oocytes recovered or the fertilization, implantation and pregnancy rates, although patients with high serum LH levels during stimulation showed significantly higher serum estradiol levels on the day of hCG.23 A more recent study involving 750 patients reported that endogenous LH levels remain sufficiently high in a GnRH antagonist protocol to support treatment with rFSH alone.32

Conclusions
To date, most trials that have examined the impact of endogenous LH levels on clinical outcomes in ART have determined that low serum concentrations do not adversely affect clinical outcomes in GnRH antagonist protocols with rFSH stimulation.29 A study by Kolibianakis and colleagues,20 however, reported that increasing LH levels had a deleterious impact on ongoing pregnancy rates and implantation rates, while a second study found that no pregnancies occurred when the change from baseline LH concentration increased or decreased dramatically during antagonist cycles.22

The Role of Supplemental LH in COS
Hypogonadotropic Hypogonadal Women

Hypogonadotropic hypogonadism is a rare heterogeneous disorder characterized by abnormally low serum FSH and LH levels and thus negligible estrogen production by the ovary. The two-cell, two-gonadotropin hypothesis predicts that both FSH and LH are needed for adequate production of estradiol by the developing follicle. An early prospective study compared the efficacy of hMG, which contains both LH and FSH activity, with that of purified FSH for inducing ovulation in 9 women with hypogonadotropic hypogonadism.33 Patients served as their own controls and were assigned in nonrandom order to stimulation with hMG (FSH 75 IU/LH 75 IU) in the first cycle and purified FSH (75 IU, LH < 1.0 IU) in the second cycle. Compared with patients treated with hMG, those treated with purified FSH experienced a statistically significant reduction in the number of leading follicles, estradiol concentrations and endometrial thickness. Eight of 9 patients (89%) in the hMG treatment cycle fulfilled the criteria for established ovulation, whereas only 3 (33%) achieved this end point after stimulation with purified FSH.33 These findings support the theory that both gonadotropins are required for appropriate ovarian steroidogenesis, follicular maturation and endometrial proliferation. Treatment with hMG containing LH activity proved clearly superior to FSH alone in this select group of patients.33

Conclusions
Studies in women with hypogonadotropic hypogonadism have demonstrated that FSH alone can induce follicular growth but that the follicles are developmentally deficient, produce abnormally low amounts of estradiol and are unable to luteinize and
rupture in response to stimulation with hCG. LH activity is required for adequate steroidogenesis, ovulation, fertilization and implantation, and in most patients with this disorder the serum LH levels are too low to support optimal follicular development. Treatment with hMG containing LH activity has proved to be clearly superior to highly purified FSH alone in this select group of patients.33

Is There a Role for Exogenous LH in Normogonadotropic Women Undergoing ART?

In recent years the complex intrafollicular synergism of FSH and LH in follicular development and steroid biosynthesis has become better understood, but the incremental clinical benefit of exogenous LH activity administered to normogonadotropic women in the early follicular phase of an ovarian stimulation cycle to enhance FSH sensitivity and improve pregnancy rates remains questionable. Thus far, no general consensus has been achieved about the lower limit of LH, below which a compromised outcome is observed. While LH is required for normal hormone production and normal oocyte and embryo growth and maturation, follicular responses to this gonadotropin in IVF cycles may depend not only on the adequacy of residual endogenous LH concentrations to sustain folliculogenesis and steroid biosynthesis following pituitary downregulation, but also on the particular stage of follicular development.

LH Supplementation in Short GnRH Agonist (“Flare-Up”) Protocol

The GnRH short, or “flare-up,” protocol combines treatment with a GnRH agonist, which is started on cycle day 2, with FSH therapy that is initiated on the same day or 1 day later. Both the agonist and FSH are usually continued until the day of hCG administration. The short protocol is designed to take advantage of an initial “flare-up” response that occurs within the first days of GnRH agonist administration and that triggers an FSH/LH surge from the pituitary before the onset of downregulation. It is theorized that the immediate stimulatory action of the GnRH agonist can jump-start, or flare up, follicular recruitment, resulting in greater quantities of mature follicles and oocytes. The short agonist protocol is often used in patients considered to be at risk of a poor ovarian response due to advanced reproductive age, an elevated FSH level, low antral follicle counts, or other signs of reduced ovarian reserve.

A recent prospective randomized trial explored whether the addition of rLH might improve the clinical outcome in a group of older, poor-responder patients undergoing their first IVF cycle in a short GnRH agonist protocol. The 84 patients in the trial were identified as poor responders based on their age (≥40 years) and elevated 3-day FSH level (≥10 mIU/mL).34 The primary outcome measures were the ongoing pregnancy rate per started cycle and the implantation rate per embryo transferred. Secondary outcomes included the number of developed follicles, number of retrieved oocytes, number of transferred embryos and several other ovarian stimulation parameters.34

All patients were treated with a fixed daily dose of 375 IU rFSH starting on day 2 of a natural cycle in a 14-day GnRH agonist “flare-up” protocol. On stimulation day 7, patients were randomized to receive 150 IU of rLH through day 14, with downward titration to half dose permitted from stimulation day 11 to day of hCG administration (group A), while those in group B continued treatment with adjustable-dose rFSH alone. The overall pregnancy rate per oocyte retrieval was 25.0% (26.3% group A; 23.7% group B), with a pregnancy wastage rate of 30.0% in group A and 22.2% in group B. No statistically significant differences were detected in any of the primary or secondary end points between the 2 treatment groups.34

Thus, the addition of rLH in the late follicular phase did not produce larger sized differences in pregnancy rates per started cycle in a group of patients classified as poor responders; however, the study is too small for confident conclusions to be drawn. The short agonist protocol adopted in this trial, however, was unlikely to have produced profound LH suppression, and this factor may have contributed to the lack of a significant LH effect in this group of older patients.34

LH Supplementation in Long GnRH Agonist Protocol

The long GnRH agonist protocol is accepted as an established treatment strategy for COS in young normogonadotropic women. Pituitary downregulation with GnRH agonists prevents the onset of a spontaneous LH surge and modulates endogenous LH activity in such a way that residual circulating levels of LH are adequate to support multiple follicular growth and oocyte development, even in the presence of FSH preparations without LH.

In a long protocol, the GnRH agonist is initiated either in the mid-luteal phase of the preceding men-
strual cycle or in the early follicular phase of the current cycle until the day of hCG administration. Ovarian stimulation with gonadotropins such as rFSH commences after pituitary suppression has been achieved.25

Evidence from Meta-analyses
In 2007, 2 meta-analyses were published that reviewed randomized controlled trials comparing the outcomes of patients treated with either rFSH alone or rFSH in combination with rLH. In the majority of the trials included in these meta-analyses, the patients underwent downregulation with a long GnRH agonist protocol. These meta-analyses have failed to generate evidence in support of statistically significant differences in pregnancy outcomes after supplementation with exogenous LH.

A meta-analysis by Kolibianakis et al35 summarized the available evidence from 7 randomized clinical trials with a total of 701 patients that assessed the impact of exogenous LH in COS cycles on live birth rate per randomized patient (Table II). Five of the trials involved the long GnRH agonist protocol, and 2 used a GnRH antagonist protocol.15,36-41 Secondary outcome variables included clinical pregnancy rate, gonadotropin consumption per cycle, duration of stimulation per cycle, estradiol and progesterone levels on day of hCG, number of cumulus-oocyte complexes (COCs) retrieved per cycle, fertilization rate per cycle and number of 2 pronuclear (2PN) oocytes per cycle.35

The analysis found that there was no significant difference in the probability of live birth with or without the addition of rLH to FSH (OR 0.92; CI 0.65–1.31; p = 0.65). This finding remained stable in all subgroup analyses, and there was no significant heterogeneity between the trials that were analyzed. In subgroup analyses, the studies were ordered by dose of rLH, timing of rLH supplementation, age of patients analyzed, type of allocation concealment and calculated or reported live birth rate.35

In the 5 studies that reported information on both

<table>
<thead>
<tr>
<th>Study</th>
<th>GnRH analogue; protocol/dose</th>
<th>FSH type in control group; starting dose; dose titration</th>
<th>Gonadotropin treatment regimen in study group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sills et al16</td>
<td>Long luteal; Leuprolin 1 or 0.5 mg/day s.c.</td>
<td>HP-FSH 150–450 IU Yes: step down</td>
<td>HP-FSH as in control group + fixed-dose, 75 IU rLH daily from start of stimulation</td>
</tr>
<tr>
<td>Balasch et al15</td>
<td>Long luteal; Leuprolin 1 or 0.5 mg/day s.c.</td>
<td>rFSH 450 IU Yes: step down</td>
<td>rFSH as in control group + fixed-dose 75 IU rLH daily from start of stimulation</td>
</tr>
<tr>
<td>Humaidan et al37</td>
<td>Long luteal; Buserelin 0.5 or 0.2 mg/day s.c.</td>
<td>rFSH 150–300 IU Yes: from S8 onward prn</td>
<td>rLH in 2:1 ratio from S8 onward</td>
</tr>
<tr>
<td>Sauer et al38</td>
<td>Pretreatment: OCP + single dose Cetrorelix 3 mg s.c.</td>
<td>rFSH 225 IU Yes: from S6 onward prn</td>
<td>rFSH as in control group + fixed-dose 150 IU rLH from start of GnRH antagonist</td>
</tr>
<tr>
<td>Griesinger et al39</td>
<td>Cetrorelix 0.25 mg/day Start: day 6</td>
<td>rFSH 150 IU Yes: from S6 onward prn</td>
<td>rFSH as in control group + 75 IU rLH, dose adjustments from S6 per rFSH dose titration</td>
</tr>
<tr>
<td>Tarlatzis et al40</td>
<td>Long follicular; Buserelin 0.2 mg/day s.c.</td>
<td>rFSH 150 IU Yes: from S6 onward prn</td>
<td>rFSH as in control group + fixed-dose 75 IU rLH daily when lead follicle at 14 mm</td>
</tr>
<tr>
<td>Fábregues et al41</td>
<td>Long luteal; Triptorelin 0.1 or 0.05 mg/day s.c.</td>
<td>rFSH 450 IU Yes: step down</td>
<td>rFSH as in control group + fixed-dose 150 IU from S6 onward</td>
</tr>
</tbody>
</table>

E2 = estradiol, ET = embryo transfer, FSH = follicle-stimulating hormone, GnRH = gonadotropin-releasing hormone, hCG = human chorionic gonadotropin, HP-FSH = highly purified follicle-stimulating hormone, ICSI = intracytoplasmic sperm injection, IVF = in vitro fertilization, LPS = luteal phase support, rLH = recombinant luteinizing hormone, n.s. = not stated, OCP = oral contraceptive pill, prn = as needed, rhCG = recombinant hCG, rFSH = recombinant follicle-stimulating hormone, S (S6, S8) = stimulation day, s.c. = subcutaneous.

clinical pregnancy and live birth rates, the addition of rLH to rFSH stimulation showed no statistically significant association with the probability of either clinical pregnancy or live birth. The number of COCs retrieved also showed no significant difference between the rFSH-alone group and the rLH plus rFSH group. In addition, the fertilization rate and the mean number of 2PN oocytes did not differ significantly between the 2 groups.

These findings should, however, be interpreted with caution because the optimal sample size to exclude clinically significant differences had not been reached by far in this meta-analysis. Nevertheless, the available data do not support the idea that the addition of rLH increases the live birth rate in patients treated with FSH and GnRH analogues for IVF.

In a second 2007 meta-analysis published in the Cochrane library, 14 studies were identified that fulfilled the meta-analysis inclusion criteria (Table III). Of these, 11 evaluated 2,396 women in GnRH agonist downregulated IVF/ICSI cycles. Ten of these 11 trials used a long agonist protocol, and 1 employed a short flare-up agonist protocol. Three studies, with a total of 216 patients, compared coadministration of rLH and rFSH to rFSH alone in a GnRH antagonist protocol.

Only 2 trials included in the meta-analysis, both employing long GnRH agonist protocols and with a combined study population of 222 women, assessed live birth rate. No evidence of a statistical difference in live birth rate was found between the rFSH alone and the rFSH plus rLH treatment groups when the data from the 2 studies were pooled. However, there was a significant heterogeneity in live birth rates between the 2 studies, and the populations in the 2 studies were also heterogeneous, with 1 study including only poor responders and the other expressly excluding poor responders.

There was no evidence of a statistical difference

<table>
<thead>
<tr>
<th>hCG</th>
<th>Criteria for hCG</th>
<th>Fertilization</th>
<th>ET day</th>
<th>LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 10,000 IU hCG</td>
<td>≥2 follicles ≥ 17 mm</td>
<td>IVF or ICSI</td>
<td>3</td>
<td>Progesterone</td>
</tr>
<tr>
<td>5,000 IU hCG</td>
<td>Consistent E2 rise in presence of ≥2 follicles ≥ 18 mm</td>
<td>IVF or ICSI</td>
<td>n.s.</td>
<td>hCG</td>
</tr>
<tr>
<td>10,000 IU hCG</td>
<td>≥3 follicles ≥ 17 mm</td>
<td>IVF or ICSI</td>
<td>2, 3 or 5</td>
<td>Progesterone</td>
</tr>
<tr>
<td>250 mcg rhCG</td>
<td>≥1 follicle ≥ 18 mm + ≥2 follicles ≥ 16 mm + E2 ~150 pg/mL per mature follicle</td>
<td>ICSI</td>
<td>n.s.</td>
<td>Progesterone</td>
</tr>
<tr>
<td>20 mcg rhCG</td>
<td>Mature follicle; 3 follicles ≥ 18 mm</td>
<td>IVF or ICSI</td>
<td>2</td>
<td>Progesterone</td>
</tr>
<tr>
<td>10,000 IU hCG</td>
<td>≥2 follicles &gt;17 mm</td>
<td>IVF or ICSI</td>
<td>2</td>
<td>Progesterone</td>
</tr>
<tr>
<td>250 mcg hCG</td>
<td>≥2 follicles ≥ 18 mm + ≥4 follicles ≥ 14 mm + consistent rise in E2</td>
<td>IVF or ICSI</td>
<td>2 or 3</td>
<td>Progesterone</td>
</tr>
</tbody>
</table>
### Table III  Randomized Controlled Trials Included in Meta-analysis by Mochtar et al42

<table>
<thead>
<tr>
<th>Study</th>
<th>GnRH analogue; protocol/dose</th>
<th>FSH type in control group; starting dose; dose titration</th>
<th>Gonadotropin treatment regimen in study group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balasch et al15</td>
<td>Long luteal; Leuprolin 1 or 0.5 mg/day s.c.</td>
<td>rFSH 450 IU Yes: step down</td>
<td>rFSH as in control group + fixed-dose 75 IU rLH daily from start of stimulation</td>
</tr>
<tr>
<td>Marrs et al43</td>
<td>Long luteal; Leuprolide 0.5mg</td>
<td>rFSH 225 IU Yes: from S6 onward prn</td>
<td>rFSH as in control group + fixed-dose 150 IU rLH from S6 onward</td>
</tr>
<tr>
<td>Humaidan et al17</td>
<td>Long luteal; Buserelin 0.5 or 0.2 mg/day s.c.</td>
<td>rFSH 150–300 IU Yes: from S8 onward prn</td>
<td>rFSH as in control group + rLH in 2:1 ratio from S8 onward</td>
</tr>
<tr>
<td>Sauer et al38</td>
<td>Pretreatment: OCP + single dose Cetorelix 3 mg s.c.</td>
<td>rFSH 225 IU Yes: from S6 onward prn</td>
<td>rFSH as in control group + fixed-dose 150 IU rLH from start of GnRH antagonist</td>
</tr>
<tr>
<td>Ferraretti et al44</td>
<td>Long luteal; GnRH agonist</td>
<td>rFSH &lt;30 yr: 150 IU 30–37 yr: 225 IU ≥38 yr: 300 IU No</td>
<td>Group A: increased dose of rFSH (max. 450 IU/day) Group B: increased dose of rFSH + 75–150 IU rLH Group C: additional FSH and LH using hMG</td>
</tr>
<tr>
<td>De Placido et al45</td>
<td>Long protocol; Triptorelin depot 3.75 mg</td>
<td>rFSH 225 IU No</td>
<td>Group A: rFSH as in control group + fixed-dose 150 IU rLH daily from S8 onward Group B: increase in FSH dose by 150 IU/day from S8 onward</td>
</tr>
<tr>
<td>Lisi et al46</td>
<td>Long luteal; Triptorelin 0.1 mg</td>
<td>rFSH 150 IU Yes: from S7 onward prn</td>
<td>Group 1: rFSH as in control + fixed-dose 37.5 IU rLH from S7 onward Group 2: rFSH as in control + fixed-dose 75 IU rLH from S7 onward</td>
</tr>
<tr>
<td>Griesinger et al19</td>
<td>Cetorelix 0.25 mg/day Start: day 6</td>
<td>rFSH 150 IU Yes: from S6 onward prn</td>
<td>rFSH as in control group + 75 IU rLH, dose adjustments from S6 per rFSH dose titration</td>
</tr>
<tr>
<td>Tarlatzis et al40</td>
<td>Long follicular; Buserelin 0.2 mg/day s.c.</td>
<td>rFSH 150 IU Yes: from S6 onward prn</td>
<td>rFSH as in control group + fixed-dose 75 IU rLH daily when lead follicle at 14 mm</td>
</tr>
<tr>
<td>Fábregues et al41</td>
<td>Long luteal; Triptorelin 0.1 or 0.05 mg/day s.c.</td>
<td>rFSH 450 IU Yes: step down</td>
<td>rFSH as in control group + fixed-dose 150 IU from S6 onward</td>
</tr>
<tr>
<td>Abdelmassih et al47</td>
<td>Long luteal; GnRH agonist</td>
<td>rFSH 225 IU Yes: from S6 onward prn</td>
<td>rFSH as in control group + fixed-dose 75 IU rLH from S7 onward</td>
</tr>
<tr>
<td>Levi-Setti et al48</td>
<td>Pretreatment: OCP Cetorelix 0.25 mg/day s.c. Start: when follicles reached 14–15 mm</td>
<td>rFSH 225 IU No</td>
<td>rFSH dose reduced to 150 IU, and rLH added in a dose of 75 IU from start of GnRH antagonist</td>
</tr>
<tr>
<td>Andersen et al49</td>
<td>Intranasal nafarelin 200 mg 3x/day for at least 2 weeks</td>
<td>rFSH fixed dose for first 6 days &lt;35 yr: 150 IU &gt;35 yr: 225 IU Yes: from S6 onward prn</td>
<td>rFSH as in control group + fixed-dose rLH (&lt;35 yr: 75 IU; &gt;35 yr: 150 IU) from S6 onward</td>
</tr>
<tr>
<td>Barrenetxea et al50</td>
<td>Short, flare-up GnRH agonist protocol</td>
<td>rFSH 375 IU</td>
<td>rFSH as in control group + rLH (dose n.s.) from S7 onward</td>
</tr>
</tbody>
</table>

E₂ = estradiol, ET = embryo transfer, FSH = follicle-stimulating hormone, GnRH = gonadotropin-releasing hormone, hCG = human chorionic gonadotropin, ICSI = intracytoplasmic sperm injection, IVF = in vitro fertilization, LH = luteinizing hormone, LPS = luteal phase support, n.s. = not stated, OCP = oral contraceptive pill, prn = as needed, rhCG = recombinant hCG, rFSH = recombinant follicle-stimulating hormone, rLH = recombinant luteinizing hormone, S (S6, S8) = stimulation day, s.c. = subcutaneous.
<table>
<thead>
<tr>
<th>hCG</th>
<th>Criteria for hCG</th>
<th>Fertilization</th>
<th>ET day</th>
<th>LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>5,000 IU hCG</td>
<td>Consistent E₂ rise in presence of ≥2 follicles &gt; 18 mm</td>
<td>IVF or ICSI</td>
<td>n.s.</td>
<td>hCG</td>
</tr>
</tbody>
</table>
| 10,000 IU hCG | ≥1 follicle ≥18 mm + ≥2 follicles  
≥16 mm + E₂ ~150 pg/mL per mature follicle | ICSI          | 2 or 3 | Progesterone|
| 10,000 IU hCG | ≥3 follicles ≥17 mm                                                            | IVF or ICSI   | 2, 3 or 5 | Progesterone|
| 250 mcg rhCG  | ≥1 follicle ≥18 mm + ≥2 follicles  
≥16 mm + E₂ ~150 pg/mL per mature follicle | ICSI          | n.s.   | Progesterone|
| 7,500 IU hCG  | n.s.                                                                           | IVF or ICSI   | 3      | Progesterone|
| 10,000 IU hCG | 3 follicles ≥17 mm                                                            | IVF or ICSI   | n.s.   | Progesterone|
| 10,000 IU hCG | n.s.                                                                           | IVF or ICSI   | n.s.   | Progesterone|
| 20 mcg rhCG   | Mature follicle; 3 follicles ≥18 mm                                              | IVF or ICSI   | 2      | Progesterone + hCG |
| 10,000 IU hCG | ≥2 follicles > 17 mm                                                            | IVF or ICSI   | 2      | Progesterone|
| 250 mcg hCG   | ≥2 follicles ≥18 mm + ≥4 follicles  
≥14 mm + consistent rise in E₂ | IVF or ICSI   | 2 or 3 | Progesterone|
| hCG (dose n.s.) | n.s.                                                                          | IVF or ICSI   | n.s.   | Progesterone + estradiol |
| 10,000 IU hCG | ≥3 follicles ≥17 mm                                                            | ICSI          | 3      | Progesterone|
| hCG (dose n.s.) | n.s.                                                                          | IVF or ICSI   | n.s.   | n.s.        |
| hCG (dose n.s.) | n.s.                                                                          | IVF           | n.s.   | n.s.        |
in clinical pregnancy rates between coadministration of rLH and rFSH compared with rFSH alone in the 7 randomized trials using a GnRH agonist in which this clinical outcome was reported. However, in 3 trials employing GnRH agonists that enrolled only poor responders,44,45,50 the pooled estimate of ongoing pregnancy was significantly higher in women co-treated with rLH compared with those treated with rFSH alone. However, these 3 studies had been excluded from the previously discussed meta-analysis35 for various methodological problems, which are described below.

It was concluded that there was a need for further large, randomized controlled trials comparing LH supplementation with rFSH alone in long GnRH agonist protocols since pooled pregnancy estimates suggest that poor responders or older women at higher risk of spontaneous abortion may benefit from co-treatment with rLH to prevent early pregnancy loss.42

Evidence from Subsequent RCT Using GnRH Long Protocol

Since publication in 2007 of the 2 meta-analyses described above, Nyboe Andersen et al51 completed a large, multicenter, multinational, randomized controlled trial to further address the issue of whether addition of rLH to rFSH in the late follicular phase enhanced ongoing pregnancy rates in a long GnRH agonist protocol. This study enrolled 526 normogonadotropic women < 40 years of age who were undergoing their first, second or third IVF/ICSI cycle. The patients were randomized in a 1:1 ratio to rFSH monotherapy or to coadministration of rLH after day 6.51 The primary efficacy end point was the number of ongoing pregnancies at weeks 10–12 per started stimulation cycle. After pituitary downregulation with a long GnRH agonist protocol, on day 1 of stimulation patients ≤ 35 years of age were allocated to rFSH 150 IU/day, and those >35 years to 225 IU/day for 6 days. After day 6 those in the control arm were randomized to continue individualized dose titration of rFSH. Those in the treatment arm were randomized to receive rLH starting on day 6, with women ≤35 years of age assigned a fixed dose of 75 IU/day, and women >35 years a fixed dose of 150 IU/day. Serum LH levels were measured on days 2–5 of the cycle, days 1 and 6 of stimulation and on day of hCG administration.51

No statistically significant differences were detected between the control and treatment arms with respect to the predefined primary end point of ongoing pregnancy rate at 10–12 weeks’ gestation (28.7% after rFSH monotherapy and 27.2% after combined rLH + rFSH). In addition, ongoing pregnancy rates in patients with low LH levels (<33rd percentile) on days 1 and 6 of stimulation showed no difference between the group treated with rFSH alone compared with the group treated with rFSH plus rLH.51

Although this study did not reach the planned sample size of 800, which was needed to give a power of 77% and a p value of <0.05, the fact that a trend toward higher ongoing pregnancy rates at weeks 10–12 was reported in the control arm of the study suggests that adding exogenous LH to the late follicular phase of IVF/ICSI cycles cannot be justified as standard practice in normogonadotrophic women <40 years of age undergoing downregulation with a long GnRH agonist protocol.51

Conclusions

Two meta-analyses published in 2007 did not generate evidence in support of any statistically significant differences in pregnancy outcomes in GnRH agonist protocols supplemented with exogenous LH compared with those stimulated with rFSH alone.35,42

The meta-analysis by Kolibianakis et al,35 which included 5 randomized controlled trials that employed GnRH long agonist protocols, found no significant difference in the probability of live birth with or without the addition of rLH to FSH, and this finding remained stable in all subgroup analyses. The authors concluded that the available data suggest that the addition of rLH does not increase the live birth rate in patients treated with FSH and GnRH analogues for IVF.

Another large meta-analysis by Mochtar et al,42 which included 10 randomized controlled trials that employed a GnRH long agonist protocol and 1 trial using a short GnRH agonist protocol, reported no statistical difference in live birth rate, clinical pregnancy rate, or ongoing pregnancy rate between the groups treated with rFSH plus LH and those treated with rFSH alone. Mochtar et al42 did point out, however, that in 3 trials employing GnRH agonists that enrolled only poor responders, the pooled estimate of ongoing pregnancy was significantly higher in women co-treated with rLH compared with those treated with rFSH alone. These findings suggest that poor responders or older women at higher risk of spontaneous abortion may benefit from rLH supplementation; therefore the authors
concluded that further large, randomized controlled trials comparing LH supplementation with rFSH alone in long GnRH agonist protocols were needed to test this possibility.

It should be noted that the 3 trials in poor responders that were included in the analysis by Mochtar et al. were not included in the meta-analysis by Kolibianakis et al. The Kolibianakis meta-analysis excluded the studies by Ferraretti et al. and De Placido et al. because in both cases the dose of rFSH had been modified at the time of initiation of rLH, while the Barrenetxea et al. study was not included because it was only available as an abstract. Thus, the Kolibianakis meta-analysis did not draw the same conclusions concerning the possible benefits of rLH supplementation in poor responders as the analysis by Mochtar et al.

Finally, a recent large, multinational, randomized controlled trial that addressed this issue concluded that supplementing rFSH with rLH during the second half of the follicular phase showed no evidence of increasing the ongoing pregnancy rates in the general population. In conclusion, it is important when comparing outcomes across randomized controlled trials to appreciate the respective objectives predefined within each study so as to reach legitimate conclusions with respect to the use versus nonuse of exogenous LH in long GnRH agonist protocols for IVF/ICSI. Further large, randomized controlled clinical trials should be undertaken, particularly in long GnRH agonist downregulation protocols, to confirm what beneficial effect, if any, rLH co-treatment imparts to those at risk for early pregnancy loss and in poor responder subgroups. Additional data from such large clinical trials could also help establish the following parameters: the threshold LH concentration needed to support folliculogenesis, steroidogenesis and oocyte maturation; how LH bioactivity correlates with absolute serum levels of LH; and what might be the impact of LH supplementation and rising estradiol levels on the bioactivity of residual LH.

LH Supplementation in GnRH Antagonist Protocols

The use of GnRH antagonists to prevent a premature LH surge in ovarian stimulation regimens is widespread, and these agents offer several potential benefits. They are associated with a reduction in the duration of therapy and gonadotropin requirement, an absence of estrogen deprivation symptoms, and a more patient-friendly protocol. Although more than 200 clinical trials involving GnRH antagonists in IVF have now been published, only a few of these studies have examined the potential role of exogenous LH in GnRH antagonist protocols. The meta-analysis by Mochtar et al. included 3 randomized controlled trials in which rFSH alone was compared with the combination of rFSH plus rLH in a total study population of 216 patients undergoing COS in a GnRH antagonist protocol. The dose and timing of administration of exogenous LH differed somewhat in these 3 trials. In 1 study, rLH was given in a dose of 150 IU on days 7–10. In the second study, rLH was given in an initial dose of 75 IU, starting on day 2; in patients in whom rFSH was increased to 300 IU on day 6, the dose of rLH was concomitantly raised to 150 IU. In the third study, rLH was given in a dose of 75 IU, starting on the day on which the antagonist was administered.

No difference in the clinical pregnancy rate was observed between the rFSH alone and the rLH co-treated groups in the 1 trial that reported on this outcome. Similarly, when the data were pooled from the 2 trials that reported on ongoing pregnancy, there was no evidence of a statistical difference between the 2 groups. Two trials reported data on the number of miscarriages, and again there was no statistical difference when the data were pooled. All 3 trials reported on the amount of retrieved oocytes, and there was no evidence of a statistical difference between the rFSH-alone group and the combined rFSH and rLH group when the data from the studies were pooled.

Conclusion

In general, supplementation with rLH did not appear to confer any significant additional benefit compared with rFSH alone on birth rate, clinical pregnancy rate, ongoing pregnancy rate or amount of retrieved oocytes in patients undergoing suppression in GnRH antagonist protocols. However, the total study population in these 3 trials was relatively small, and there is a need for additional large, randomized, well-controlled trials as well as trials performed in patient populations such as poor responders or patients of advanced maternal age.

hMG Versus rFSH for Ovarian Stimulation

An issue that has been examined in several meta-analyses and randomized controlled trials published since 2006 is whether the use of rFSH alone or hMG for ovarian stimulation produces any significant differences in pregnancy rate and other clinical out-
comes in patients treated with either a long GnRH agonist protocol or a GnRH antagonist protocol.

GnRH Agonist Protocol

Two recent meta-analyses examined evidence from randomized trials comparing the impact of hMG versus rFSH on live birth rates in women undergoing COS in IVF/ICSI cycles. The meta-analysis by Al-Inany et al included 12 randomized trials involving 3,575 women: 9 trials were long GnRH agonist protocols, 1 was a short GnRH agonist protocol, 1 involved a GnRH antagonist protocol, and in 1 trial the patients did not undergo pituitary downregulation. The meta-analysis concluded that the live birth rate was significantly higher in the women treated with hMG compared with rFSH (OR 1.20, 95% CI 1.01–1.42) and that the rates of ovarian hyperstimulation syndrome did not differ significantly between the 2 groups.

The second meta-analysis by Coomarasamy et al evaluated the efficacy of hMG versus rFSH in women undergoing IVF or ICSI following a long GnRH agonist protocol, with live birth rate as the primary outcome. This study, which analyzed the results of 7 randomized trials that enrolled 2,159 women, demonstrated a small yet significant increase in the live birth rate with hMG compared with rFSH (RR 1.18, 95% CI 1.02–1.38; p = 0.03). Thus, treatment with hMG was associated with a 4% increase in live birth rate compared with rFSH in IVF or ICSI treatment following a long agonist downregulation protocol.

A third meta-analysis evaluated 6 trials with 2,371 participants in which the efficacy of highly purified hMG (HP-hMG) was compared with that of rFSH in women undergoing IVF or ICSI. HP-hMG was found to be associated with a higher ongoing pregnancy/live birth rate (OR 1.31) compared with rFSH only in IVF studies, whereas in ICSI procedures no significant difference was detected (OR 0.98). It was concluded that HP-hMG, compared with rFSH, was associated with better treatment outcomes only in IVF cycles and not in ICSI cycles. Although the reason for the different effects of HP-hMG in IVF versus ICSI cycles is not completely understood, Al-Inany and colleagues suggest that it may be related to the beneficial effect of maintaining the cumulus-oocyte complexes intact in IVF compared with ICSI cycles.

The MERIT study, which was conducted in a group of 731 patients, found that a higher proportion of oocytes developed into top-quality embryos in patients treated with HP-hMG (11.3%) compared with rFSH (9.0%; p = 0.044). In a follow-up study in the same group of patients, Smitz et al showed that at the end of stimulation, serum estradiol was higher with HP-hMG (p = 0.031), whereas progesterone was higher with rFSH (p < 0.001). Most of the LH activity in the HP-hMG preparation used in this study derived from hCG, and it appears that hCG induced paracrine factors that led to high estrogen levels at the end of stimulation in the group treated with HP-hMG. Conversely, the study authors suggested that progesterone levels were higher in the rFSH group because FSH-stimulated granulosa cells produce paracrine factors that either stimulate progesterone production or decrease the action of enzymes that normally suppress the conversion of progesterone to androgens. Thus, exogenous LH activity (supplied by HP-hMG) induces a differential endocrine environment compared with rFSH alone, but the ultimate impact of such differences on the clinical outcome is still unknown.

Conclusions

Three recent meta-analyses concluded that the use of hMG is associated with a small but statistically significant increase in live birth rates and clinical pregnancy rates compared with rFSH among women undergoing ART in long GnRH agonist protocols. However, the most recent meta-analysis by Al-Inany et al found that this beneficial effect of hMG was present only in IVF trials and not in ICSI studies.

Although exogenous LH activity (supplied by HP-hMG) has been shown to induce a differential endocrine environment compared with rFSH, the possible impact of such differences on clinical outcomes in IVF procedures has not yet been determined.

GnRH Antagonist Protocol

Until recently, only limited data have been available comparing rFSH and hMG in women being treated with a GnRH antagonist protocol. A 2008 randomized controlled trial addressed this issue by comparing HP-hMG with rFSH in 280 women undergoing IVF/ICSI using a GnRH antagonist protocol that defined ongoing pregnancy rate as the primary end point. No significant differences were observed between the HP-hMG and rFSH groups in the ongoing pregnancy rate per started cycle, or for rates of implantation, clinical pregnancy or pregnancy loss. However, more oocytes were obtained from patients treated with rFSH than with HP-hMG.
(14.4 ± 8.1 vs. 11.3 ± 6.0; p = 0.001). Estradiol levels were higher at the end of stimulation in the HP-hMG group, while progesterone levels were higher in the rFSH group. Thus, although some differences were noted in the oocyte yield and hormonal profile in the 2 groups, patients treated with HP-hMG or rFSH in a GnRH antagonist protocol had similar ongoing pregnancy rates per started cycle (35.0 vs. 32.1%, respectively).58

**Conclusions**

In the first randomized controlled trial comparing HP-hMG and rFSH in the GnRH antagonist protocol, the ongoing pregnancy rate per started cycle was similar in the 2 groups.58 However, these findings are limited because of the relatively small number of patients, and this study only supports the conclusion that there is an absence of a large difference between HP-hMG and rFSH in cycle outcome. Large multicenter analyses or meta-analyses of several single studies are needed to determine whether smaller differences (e.g., 10%) in clinical outcome might exist between the 2 compounds in a GnRH antagonist protocol.

**ART Subpopulations and Clinical Response to LH Supplementation**

At this point, evidence has been presented indicating a clear benefit for LH supplementation in hypogonadal hypogonadotropic women. In general, however, evidence supporting the routine use of LH supplementation in long GnRH agonist protocols is inconclusive, and there is no strong evidence to support its use in GnRH antagonist protocols. Now, we will assess what the evidence says about the possible value of LH add-back in specific subpopulations of women.

Some evidence suggests that exogenous supplementation of LH activity may offer added clinical benefits to certain ART subgroups, e.g., women of advanced reproductive age, those who have an initial slow response to gonadotropin stimulation or require excessively high doses of FSH to complete follicular development, and women with deeply suppressed LH levels after treatment with long GnRH agonist protocols. The available studies, however, are mostly small in size and often of low methodological quality.43,59

**Women of Advanced Reproductive Age**

According to the Practice Committee of the American Society for Reproductive Medicine, it is generally recognized that infertility becomes more pronounced after age 35, despite evidence of continued, regular ovulatory cycles. Various alternative ART protocols have been implemented in an attempt to overcome the age-related decrease in ovarian responsiveness to gonadotropins, including adjusting the dose and composition of gonadotropin preparations, use of microdose GnRH agonist protocols, GnRH antagonist protocols with FSH, or low-dose GnRH agonist pituitary suppression prior to gonadotropin stimulation.

Several randomized controlled trials evaluating the impact of LH supplementation on clinical outcomes in long GnRH agonist protocols have also assessed the effect of exogenous LH in subgroups of women of advanced reproductive age (e.g., ≥ 35 years) (Table IV). Two recent trials found that administration of exogenous rLH was associated with improved treatment outcome in women ≥ 35 years undergoing IVF or ICSI cycles,37,43 but a third trial concluded that LH supplementation did not increase the ovarian response or implantation rates in older women.41 Two of these trials37,43 met the criteria for inclusion in the recent meta-analysis by Kolibianakis et al,35 but the third study was excluded from the analysis because of a methodological problem that occurred during the study period—i.e., a significantly different number of embryos was transferred in the two arms of the study.43

**Patients Hyporesponsive to FSH in GnRH Agonist Protocol**

De Placido et al45 observed that about 12–14% of patients treated with a depot GnRH agonist respond abnormally with suboptimal increases in estradiol concentrations on days 5 and 8 of stimulation and a plateau of initial follicular growth. Despite appropriately adjusted dosage increases of FSH, a “steady response” ensues, with a large consumption of gonadotropin accompanied by a suboptimal ovarian response.45 In a recent randomized controlled trial, they assessed whether 130 “steady responders” to rFSH might be “rescued” by administering hMG as a source of exogenous LH.45

Women exhibiting a “steady response” to rFSH stimulation were randomized to either a fixed daily dose of 150 IU rLH or to an increase of 150 IU in the total daily dose of rFSH; normal responders formed a control group. The average number of cumulus oocyte complexes retrieved and the mean number of mature oocytes per ICSI cycle were significantly higher in the group that received rLH, indicating...
that exogenous LH supplementation can improve the ovarian response in young normogonadotropic women who exhibit an initial suboptimal response ("steady response") to rFSH. In another prospective randomized trial, Ferraretti et al. investigated the role of exogenous LH in a group of 126 women deemed "hyporesponsive" to ovarian stimulation with rFSH. The hyporesponsive patients were randomized into 3 groups: one group received a higher dosage of rFSH, a second group was given rLH in addition to the increased dose of rFSH, and a third group received additional FSH and LH in the form of hMG; a group of 54 age-matched normal responders served as controls.

The pregnancy and implantation rates were statistically significantly higher in the rLH group compared with the groups treated with rFSH or rFSH plus hMG and were similar to those of the normal responders. In addition, the live birth rate per started cycle was similar between the rLH group and the normal responders, while the live birth rates in the other 2 treatment groups were half as high. The results suggest that exogenous rLH supplementation in patients hyporesponsive to FSH may help improve oocyte competence and produce viable embryos.

Although these randomized controlled trials indicate a possible benefit of LH supplementation in normogonadotropic patients who show a suboptimal response to FSH, it should be noted that these studies were excluded from a recent systematic review and meta-analysis because in both cases the dose of FSH was modified at the time of initiation of LH.

### Conclusions

A number of prospective clinical trials have suggested that various clinical outcomes can be expected from exogenous LH supplementation in specific ART subgroups that are distinguished from normal responder populations by advanced reproductive age (≥ 35 years) or hyporesponsiveness to initial gonadotropin stimulation.

The data concerning the potential benefits of LH supplementation for women of advanced reproductive age are, however, inconclusive. Additional well-designed randomized controlled trials performed in similar patient populations using uniform FSH, LH and GnRH agonist formulations, doses, and administration schedules and with specific predefined clinical outcomes are needed to help resolve this issue.

Data from 2 randomized controlled trials indicate that supplemental LH may improve the ovarian response and clinical outcomes in women with an initial suboptimal response ("steady response") to ovarian stimulation. However, both of these trials were excluded from a recent meta-analysis because of methodological problems. Thus, additional well-designed randomized controlled trials are needed to determine whether supplemental LH may be beneficial in such hyporesponsive patients.

### Table IV: LH Supplementation in Women of Advanced Reproductive Age (≥ 35 Years)

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient age (yr)</th>
<th>Treatment</th>
<th>Type of COS</th>
</tr>
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<tbody>
<tr>
<td>Marrs</td>
<td>18–40 (116 patients ≥ 35 yr)</td>
<td>Long GnRH agonist protocol for ICSI</td>
<td>rFSH + rLH starting on day 6 vs. rFSH alone</td>
</tr>
<tr>
<td>Humaidan</td>
<td>22–40 (37 patients ≥ 35 yr)</td>
<td>Long GnRH agonist protocol for IVF/ICSI</td>
<td>rFSH + rLH (in a ratio of 2:1) starting on day 8 vs. rFSH alone</td>
</tr>
<tr>
<td>Fabregues</td>
<td>All patients ≥ 35 yr</td>
<td>Long GnRH agonist protocol for IVF/ICSI</td>
<td>rFSH + rLH starting on day 6 vs. rFSH alone</td>
</tr>
</tbody>
</table>

COS = controlled ovarian stimulation, GnRH = gonadotropin-releasing hormone, ICSI = intracytoplasmic sperm injection, IVF = in vitro fertilization, LH = luteinizing hormone, n.s. = not stated, rFSH = recombinant follicle-stimulating hormone, rLH = recombinant luteinizing hormone.
Discussion

LH plays a well-established role in normal folliculogenesis and ovulation by inducing the synthesis of androgens, autocrine and paracrine regulators and the enzymes necessary for progesterone production. The mid-cycle surge in LH leads to the physical act of ovulation and suppresses the inhibitors of nuclear maturation in the egg.

In patients with hypogonadotrophic hypogonadism, it has been clearly shown that although FSH alone can induce follicular growth, LH is required for adequate steroidogenesis, fertilization and implantation. Whether the low endogenous LH levels that can occur during pituitary downregulation with GnRH agonists in normogonadotropic women are sufficient to foster normal follicular maturation and development of oocyte competence is still a matter of controversy. This review has sought to determine if current evidence supports a need for supplemental LH in certain COS protocols or in particular patient populations.

Evidence from recent meta-analysis suggests that low endogenous LH levels that occur during ovarian stimulation for IVF using long GnRH agonist protocols are not associated with a decreased probability of ongoing pregnancy beyond 12 weeks. Some data indicate that very high mid-follicular endogenous LH levels may be associated with a decreased probability of a successful IVF outcome. The association of endogenous serum LH with clinical outcome has been assessed in only a few studies, however, so data from additional well-controlled trials are needed to either support or modify the conclusions drawn from this systematic review.

The use of hMG in long GnRH agonist protocols has been linked with a small but statistically significant increase in live birth rates and clinical pregnancy rates compared with rFSH, but it appears that this beneficial effect of hMG is present only in IVF cycles and not in ICSI cycles.

Evidence supporting the routine use of LH supplementation in long GnRH agonist protocols is still somewhat inconclusive, but some data suggest that exogenous LH may offer added clinical benefits to certain subgroups, such as women aged ≥ 35 years and patients with an initial suboptimal, or steady, response to gonadotropin stimulation. However, 3 of the studies supporting the use of supplemental LH in these subpopulations were excluded from a recent meta-analysis because of methodological problems.

Exogenous LH does not appear to offer any additional benefit in patients undergoing pituitary suppression in GnRH antagonist regimens. In 3 randomized controlled trials in which rFSH alone was compared with the combination of rFSH plus rLH in GnRH antagonist protocols, LH supplementation did not have any beneficial effects on birth rate, clinical pregnancy rate, ongoing pregnancy rate or number of retrieved oocytes. In addition, in a randomized controlled trial comparing

<table>
<thead>
<tr>
<th>End point</th>
<th>Finding</th>
<th>Conclusion</th>
</tr>
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<tbody>
<tr>
<td>Clinical pregnancy rate per started cycle</td>
<td>&lt;35: n.s.</td>
<td>LH does not appear to benefit patients &lt;35;</td>
</tr>
<tr>
<td>Implantation rate; FSH consumption</td>
<td>≥ 35: rFSH + rLH: 36.4%</td>
<td>Addition of exogenous rLH appears to benefit patients ≥ 35 by improving implantation rate and reducing rFSH consumption</td>
</tr>
<tr>
<td>Oocyte yield and maturity, no. of fertilized oocytes, implantation rate, clinical pregnancy rate</td>
<td>Oocyte yield and maturity, no. of fertilized oocytes: significantly higher in rFSH alone group</td>
<td>Addition of exogenous LH does not increase ovarian response or implantation rates in women ≥ 35 yr</td>
</tr>
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</table>


Addition of exogenous LH appears to benefit patients ≥ 35 by improving implantation rate and reducing rFSH consumption.
HP-hMG with rFSH in a GnRH antagonist protocol, the ongoing pregnancy rate per started cycle was similar in the 2 groups.\textsuperscript{58}

These findings, however, are limited because of the relatively small number of patients in all these trials, and more data are needed to confirm the apparent lack of benefit from LH supplementation in antagonist protocols.

The evidence seems to favor some form of LH add-back in certain well-defined groups, but such patients account for only a minority of IVF patients undergoing luteal downregulation for ovarian stimulation in long GnRH agonist protocols. The available evidence does not favor routine LH add-back for normogonadotropic women outside the special groups that have been identified. In particular, exogenous LH does not appear to offer any significant benefit to patients undergoing pituitary suppression in GnRH antagonist protocols. The use of GnRH antagonists as the basis for standard protocols would obviate the need for LH add-back and simplify decision making for the physician and the treatment regimen for the patient. GnRH antagonists offer a more patient-friendly approach because they permit a shorter duration of therapy, a reduction in gonadotropin requirements, and freedom from the estrogen-deprivation symptoms associated with long GnRH agonist protocols. What remains to be done to confirm that supplemental LH is not needed in antagonist protocols are well-controlled trials, with standardized GnRH antagonist management, that compare rFSH alone with rFSH plus HMG, rFSH plus rLH and with HMG alone.

Reproductive endocrinologists in the United States are starting to increase their use of GnRH antagonists. While there is sufficient evidence to support the efficacy of the antagonist protocol in the absence of LH add-back, this approach has not yet been universally accepted, and until it is, the controversy surrounding the routine use of LH add-back will likely continue in the United States.

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